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=> d his ful

     (FILE 'REGISTRY' ENTERED AT 11:09:55 ON 01 MAY 2006)
                DEL HIS Y
                E MD-2/CN
L1            2 SEA ABB=ON  PLU=ON  ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR)"/C
                N OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246)"/CN)

     FILE 'CAPLUS' ENTERED AT 11:12:14 ON 01 MAY 2006
L2            6 SEA ABB=ON  PLU=ON  L1
                D SCAN TI
L3          221 SEA ABB=ON  PLU=ON  (MD2/OBI OR MD 2/OBI) (L) PROTEIN#/OBI
L4        22197 SEA ABB=ON  PLU=ON  ENDOTOXIN#/OBI OR ENDO/OBI (L) TOXIN#/OBI
L5       260218 SEA ABB=ON  PLU=ON  (GRAM POS?/OBI OR GRAM NEG?/OBI) (2A)
                (BACTERIA/OBI OR COCCI/OBI OR ROD#/OBI) OR NEISSERIA/OBI OR
                ESCHERICHIA/OBI OR PSEUDOMONAS/OBI OR HAEMOPHILUS/OBI OR
                SALMONELLA/OBI OR FRANCISELLA/OBI
L6       978744 SEA ABB=ON  PLU=ON  COMPLEX?/OBI OR CONJUGAT?/OBI
L7       658945 SEA ABB=ON  PLU=ON  BOUND/OBI OR BIND###/OBI OR ATTACH###/OBI
L8           23 SEA ABB=ON  PLU=ON  L3 (L) L4
L9           11 SEA ABB=ON  PLU=ON  L8 AND ((L6 OR L7))
L10           2 SEA ABB=ON  PLU=ON  L3 (L) L5 (L) (L6 OR L7)
L11          13 SEA ABB=ON  PLU=ON  L3 AND L4 AND ((L6 OR L7))
L12          13 SEA ABB=ON  PLU=ON  L9 OR L11
L13          11 SEA ABB=ON  PLU=ON  L3 AND L5 AND ((L6 OR L7))
L14          11 SEA ABB=ON  PLU=ON  L10 OR L13
L15           8 SEA ABB=ON  PLU=ON  L14 NOT L11
L16           7 SEA ABB=ON  PLU=ON  MYELOID DIFFERENTIATION PROTEIN/OBI (2W)
                2/OBI
L17           4 SEA ABB=ON  PLU=ON  L16 AND (L4 OR L5)
L18           1 SEA ABB=ON  PLU=ON  L17 AND ((L6 OR L7))
L19           0 SEA ABB=ON  PLU=ON  L18 NOT (L15 OR L11)

     FILE 'MEDLINE' ENTERED AT 11:53:22 ON 01 MAY 2006
L20           0 SEA ABB=ON  PLU=ON  L1
L21         392 SEA ABB=ON  PLU=ON  MD2 OR MD 2
                E ENDOTOXIN/CT
                E E4+ALL
L22       18574 SEA ABB=ON  PLU=ON  ENDOTOXINS/CT
                E GRAM NEGATIVE BACTERIA/CT
                E E3+ALL
                E E2+ALL
L23        9444 SEA ABB=ON  PLU=ON  "GRAM-NEGATIVE BACTERIA"/CT
                E GRAM-POSITIVE BACTERIA/CT
L24        6212 SEA ABB=ON  PLU=ON  "GRAM-POSITIVE BACTERIA"/CT
L25      355252 SEA ABB=ON  PLU=ON  NEISSERIA OR ESCHERICHIA OR PSEUDOMONAS OR
                HAEMOPHILUS OR HEMOPHILUS OR SALMONELLA OR FRANCISELLA
L26          22 SEA ABB=ON  PLU=ON  L21 AND L22
L27      694546 SEA ABB=ON  PLU=ON  COMPLEX? OR CONJUGAT?
L28      960957 SEA ABB=ON  PLU=ON  BIND? OR BOUND OR ATTACH?
L29          12 SEA ABB=ON  PLU=ON  L28 AND L26
L30          77 SEA ABB=ON  PLU=ON  L21 AND (L23 OR L24 OR L25)
L31          28 SEA ABB=ON  PLU=ON  L30 AND L28
L32      794489 SEA ABB=ON  PLU=ON  SEQUENCE?
L33           3 SEA ABB=ON  PLU=ON  L31 AND L32
L34       56265 SEA ABB=ON  PLU=ON  ENDOTOXINS+NT/CT
L35         185 SEA ABB=ON  PLU=ON  L34 AND L21
L36          94 SEA ABB=ON  PLU=ON  L35 AND L27
               L37          23 SEA ABB=ON  PLU=ON  L21 (S) ENDOTOXIN?
L38          20 SEA ABB=ON  PLU=ON  L37 AND ((L27 OR L28))
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L39          14 SEA ABB=ON  PLU=ON  L26 AND L27
L40          24 SEA ABB=ON  PLU=ON  L39 OR L38

     FILE 'BIOSIS' ENTERED AT 12:45:25 ON 01 MAY 2006
L41           0 SEA ABB=ON  PLU=ON  L1
L42         528 SEA ABB=ON  PLU=ON  MD2 OR MD 2
L43       27499 SEA ABB=ON  PLU=ON  ENDOTOXIN?
L44          53 SEA ABB=ON  PLU=ON  L42 (L) L43
L45      698729 SEA ABB=ON  PLU=ON  COMPLEX? OR CONJUGAT?
L46          26 SEA ABB=ON  PLU=ON  L44 AND L45
L47      904058 SEA ABB=ON  PLU=ON  BIND? OR BOUND  OR ATTACH?
L48          20 SEA ABB=ON  PLU=ON  L42 (L) L47 AND L43
L49          33 SEA ABB=ON  PLU=ON  L48 OR L46

     FILE 'WPIDS' ENTERED AT 12:47:49 ON 01 MAY 2006
L50         139 SEA ABB=ON  PLU=ON  MD2 OR MD 2
L51        3135 SEA ABB=ON  PLU=ON  ENDOTOXIN? OR ENDO (2W) TOXIN#
L52           4 SEA ABB=ON  PLU=ON  L50 (S) L51
L53           5 SEA ABB=ON  PLU=ON  L50 (L) L51
L54     1289131 SEA ABB=ON  PLU=ON  BIND? OR BOUND OR ATTACH?
L55          14 SEA ABB=ON  PLU=ON  L50 (S) L54
L56           3 SEA ABB=ON  PLU=ON  L55 AND L51
L57           6 SEA ABB=ON  PLU=ON  L56 OR L53

     FILE 'BIOSIS' ENTERED AT 12:49:22 ON 01 MAY 2006
L58          25 SEA ABB=ON  PLU=ON  L42 (S) (L45 OR L47) AND L43
L59           8 SEA ABB=ON  PLU=ON  L49 NOT L58

     FILE 'CAPLUS, MEDLINE, WPIDS, BIOSIS' ENTERED AT 12:50:42 ON 01 MAY 2006
L60          50 DUP REM L11 L15 L40 L57 L58 (26 DUPLICATES REMOVED)
                ANSWERS '1-21' FROM FILE CAPLUS
                ANSWERS '22-38' FROM FILE MEDLINE
                ANSWERS '39-42' FROM FILE WPIDS
                ANSWERS '43-50' FROM FILE BIOSIS
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=> fil caplus medline wpids biosis
FILE 'CAPLUS' ENTERED AT 12:53:07 ON 01 MAY 2006
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 12:53:07 ON 01 MAY 2006

FILE 'WPIDS' ENTERED AT 12:53:07 ON 01 MAY 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE 'BIOSIS' ENTERED AT 12:53:07 ON 01 MAY 2006
Copyright (c) 2006 The Thomson Corporation

=> d que l60
L3          221 SEA FILE=CAPLUS ABB=ON  PLU=ON  (MD2/OBI OR MD 2/OBI) (L)
                PROTEIN#/OBI
L4        22197 SEA FILE=CAPLUS ABB=ON  PLU=ON  ENDOTOXIN#/OBI OR ENDO/OBI (L)
                TOXIN#/OBI
L5       260218 SEA FILE=CAPLUS ABB=ON  PLU=ON  (GRAM POS?/OBI OR GRAM
                NEG?/OBI) (2A) (BACTERIA/OBI OR COCCI/OBI OR ROD#/OBI) OR
                NEISSERIA/OBI OR ESCHERICHIA/OBI OR PSEUDOMONAS/OBI OR
                HAEMOPHILUS/OBI OR SALMONELLA/OBI OR FRANCISELLA/OBI
L6       978744 SEA FILE=CAPLUS ABB=ON  PLU=ON  COMPLEX?/OBI OR CONJUGAT?/OBI
L7       658945 SEA FILE=CAPLUS ABB=ON  PLU=ON  BOUND/OBI OR BIND###/OBI OR
                ATTACH###/OBI
L10           2 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 (L) L5 (L) (L6 OR L7)
L11          13 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L4 AND ((L6 OR L7))
L13          11 SEA FILE=CAPLUS ABB=ON  PLU=ON  L3 AND L5 AND ((L6 OR L7))
L14          11 SEA FILE=CAPLUS ABB=ON  PLU=ON  L10 OR L13
L15           8 SEA FILE=CAPLUS ABB=ON  PLU=ON  L14 NOT L11
L21         392 SEA FILE=MEDLINE ABB=ON  PLU=ON  MD2 OR MD 2
L22       18574 SEA FILE=MEDLINE ABB=ON  PLU=ON  ENDOTOXINS/CT
L26          22 SEA FILE=MEDLINE ABB=ON  PLU=ON  L21 AND L22
L27      694546 SEA FILE=MEDLINE ABB=ON  PLU=ON  COMPLEX? OR CONJUGAT?
L28      960957 SEA FILE=MEDLINE ABB=ON  PLU=ON  BIND? OR BOUND OR ATTACH?
L37          23 SEA FILE=MEDLINE ABB=ON  PLU=ON  L21 (S) ENDOTOXIN?
L38          20 SEA FILE=MEDLINE ABB=ON  PLU=ON  L37 AND ((L27 OR L28))
L39          14 SEA FILE=MEDLINE ABB=ON  PLU=ON  L26 AND L27
L40          24 SEA FILE=MEDLINE ABB=ON  PLU=ON  L39 OR L38
L42         528 SEA FILE=BIOSIS ABB=ON  PLU=ON  MD2 OR MD 2
L43       27499 SEA FILE=BIOSIS ABB=ON  PLU=ON  ENDOTOXIN?
L45      698729 SEA FILE=BIOSIS ABB=ON  PLU=ON  COMPLEX? OR CONJUGAT?
L47      904058 SEA FILE=BIOSIS ABB=ON  PLU=ON  BIND? OR BOUND  OR ATTACH?
L50         139 SEA FILE=WPIDS ABB=ON  PLU=ON  MD2 OR MD 2
L51        3135 SEA FILE=WPIDS ABB=ON  PLU=ON  ENDOTOXIN? OR ENDO (2W) TOXIN#
L53           5 SEA FILE=WPIDS ABB=ON  PLU=ON  L50 (L) L51
L54     1289131 SEA FILE=WPIDS ABB=ON  PLU=ON  BIND? OR BOUND OR ATTACH?
L55          14 SEA FILE=WPIDS ABB=ON  PLU=ON  L50 (S) L54
L56           3 SEA FILE=WPIDS ABB=ON  PLU=ON  L55 AND L51
L57           6 SEA FILE=WPIDS ABB=ON  PLU=ON  L56 OR L53
L58          25 SEA FILE=BIOSIS ABB=ON  PLU=ON  L42 (S) (L45 OR L47) AND L43
L60          50 DUP REM L11 L15 L40 L57 L58 (26 DUPLICATES REMOVED)


=> d .ca l60 1-21;d ibib ab ct l60 22-50

L60  ANSWER 1 OF 50  CAPLUS  COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER:        2006:211512  CAPLUS
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DOCUMENT NUMBER: 144:286155
TITLE: Compositions and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections
INVENTOR(S): Kirkland, Theo N., III; Viriyakosol, Sunganya
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 58 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006025995	A2	20060309	WO 2005-US26771	20050727

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.: US 2004-591805P P 20040727
US 2005-681097P P 20050513

ED Entered STN: 09 Mar 2006

AB The invention provides compns. and methods for the targeted bacteriostatic and antibacterial agents and for treatment of sepsis caused by infectious diseases, such as bacterial and fungal diseases. In one aspect, the invention provides methods and compns. for decreasing the levels of LPS in the circulation of an individual, e.g., a human patient with sepsis, e.g., gram neg. septic shock. In one aspect, the invention is directed to chimeric proteins comprising the MD-2 polypeptide and an opsonizing agent, e.g., antibody Fc domains, or equivalent In one aspect, the invention is directed to chimeric proteins comprising fragments or altered form of MD-2 polypeptide and an opsonizing agent, e.g., antibody Fc domains, or equivalent The invention also provides pharmaceutical compns. comprising the chimeric polypeptides of the invention, and methods of making and using them, including methods for ameliorating or preventing sepsis. The invention also provides compns. for transfecting cells with nucleic acid comprising the mutant MD-2 proteins and/or the chimeric polypeptides of the invention.

CC 1-5 (Pharmacology)
ST human **MD2 protein** mutant antibacterial antifungal therapy
IT Gram-negative bacteria
(-induced septic shock; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)
IT Collagens, biological studies
Fibrinogens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(-like domain; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT **Proteins**
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(C; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT **Protein** motifs
(Carbohydrate-**binding** domain; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT **Proteins**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**MD-2**; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Gene, animal
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**MD2**, mutation of; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Complement
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(activating agent; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Asthma
(acute respiratory; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Respiratory distress syndrome
(acute; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Opsonization
(agent; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Peptides, biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(antimicrobial; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Infection
(bacterial; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Fungi
Insecta
Yeast
(cell, as host; compns. and methods using human **MD-2**

mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Infection
(chronic; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Anti-infective agents
Anti-inflammatory agents
Antiasthmatics
Antibiotics
Antiviral agents
Autoimmune disease
Blood
Blood serum
Body fluid
Cerebrospinal fluid
Eubacteria
Fungicides
Genetic vectors
Granulomatous disease
Human
Immunomodulators
Mycosis
Plant cell
Plasmid vectors
Protein engineering
Protein sequences
Shock (circulatory collapse)
Transplant rejection
(compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Antibodies and Immunoglobulins
Immunoglobulin receptors
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT CD14 (antigen)
Lipopolysaccharides
Promoter (genetic element)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Cytokines
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Fusion **proteins** (chimeric **proteins**)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(comprising **protein MD-2**; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**, -induced septic shock; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Genetic element
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(expression cassette, for **protein MD-2**; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ficolin; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Antibodies and Immunoglobulins
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(fragments, Fc domain; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Transplant and Transplantation
(graft-vs.-host reaction; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Animal cell
(mammalian; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Signal peptides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(of **protein MD-2**; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Infection
(parasitic; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Antibacterial agents
(peptide, **protein**; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Shock (circulatory collapse)
(septic, **endotoxin**-induced; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Sepsis
(severe; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Shock (circulatory collapse)
(toxic shock syndrome, **endotoxin**-induced; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Injury
(trauma; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

IT Inflammation
(treatment of; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)
IT Infection
(viral; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)
IT 878298-23-8P, **Protein MD-2** (human precursor) 878298-24-9P, **Protein MD-2** (human mutant isoform) 878298-25-0P, **Protein MD-2** (human mutant isoform) 878298-26-1P, **Protein MD-2** (human mutant isoform)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; compns. and methods using human **MD-2** mutants and chimeric **proteins** for treating bacterial and fungal infections)

L60 ANSWER 2 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2005:426231 CAPLUS
DOCUMENT NUMBER: 142:480799
TITLE: Preparation of **complexes** of **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**
INVENTOR(S): Weiss, Jerrold P.; Gioannini, Theresa L.; Teghanemt, Athamane; Subramanian, Ramaswamy
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 34 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117
ED Entered STN: 19 May 2005
AB The disclosed invention provides purified water soluble complexes of endotoxin and MD-2. The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using

complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4, but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.

IC ICM A61K039-02
ICS C07K014-195
INCL 424235100; 530395000
CC 15-10 (Immunochemistry)
ST **endotoxin** MD2 **complex** TLR4 receptor cell activation
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(CD14 requirement in preparation of **complexes** of bacterial **endotoxin** and MD-2)
IT Animal cell line
(Hek 293; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT **Proteins**
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**MD-2**, **endotoxin complexes**; preparation of **complexes** of bacterial **endotoxin** and **MD-2** and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Immunostimulants
(adjuvants; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT Glycolipids
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(bacterial; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Drug delivery systems
(carriers; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Toxins
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**endotoxins**, MD-2 **complexes**; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Toxins
RL: BPN (Biosynthetic preparation); BUU (Biological use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**endotoxins**, acylated; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

IT Respiratory system
(epithelium; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT Immunity
(innate; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT Cell activation
Escherichia
Escherichia coli
Francisella
Francisella tularensis
Haemophilus
Haemophilus influenzae
Neisseria
Neisseria meningitidis
Pseudomonas
Pseudomonas aeruginosa
Salmonella
Salmonella typhimurium
(preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)
IT Anti-inflammatory agents
Human
(preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT Epithelium
(respiratory tract; preparation of **complexes** of bacterial **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin** in)
IT 852008-84-5 852009-59-7 852009-60-0 852009-61-1 852009-62-2
852009-63-3 852009-64-4 852009-65-5 852009-66-6 852009-67-7
852009-68-8 852009-69-9 852009-70-2 852009-71-3 852009-72-4
852009-73-5
RL: PRP (Properties)
(unclaimed nucleotide sequence; preparation of **complexes** of **endotoxin** and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by **endotoxin**)

L60 ANSWER 3 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2005:1207627 CAPLUS
DOCUMENT NUMBER: 143:458453
TITLE: Biochemical and Functional Characterization of Membrane Blebs Purified from **Neisseria** meningitidis Serogroup B
AUTHOR(S): Post, Deborah M. B.; Zhang, DeSheng; Eastvold, Joshua S.; Teghanemt, Athmane; Gibson, Bradford W.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine and the Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, 52242, USA
SOURCE: Journal of Biological Chemistry (2005), 280(46), 38383-38394
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 14 Nov 2005
AB Studies with purified aggregates of endotoxin have revealed the importance of lipopolysaccharide-binding protein (LBP)-dependent extraction and transfer of individual endotoxin mols. to CD14 in Toll-like receptor 4 (TLR4)-dependent cell activation. Endotoxin is normally embedded in the outer membrane of intact Gram-neg. bacteria and shed membrane vesicles ("blebs"). However, the ability of LBP and CD14 to efficiently promote TLR4-dependent cell activation by membrane-associated endotoxin has not been studied extensively. In this study, the authors used an acetate auxotroph of Neisseria meningitidis serogroup B to facilitate metabolic labeling of bacterial endotoxin and compared interactions of purified endotoxin aggregates and of membrane-associated endotoxin with LBP, CD14, and endotoxin-responsive cells. The endotoxin, phospholipid, and protein composition of the recovered blebs indicate that the blebs derive from the bacterial outer membrane. Proteomic anal. revealed an unusual enrichment in highly cationic (pI > 9) proteins. Both purified endotoxin aggregates and blebs activate monocytes and endothelial cells in a LBP-, CD14-, and TLR4/MD-2-dependent fashion, but the blebs were 3-10-fold less potent when normalized for the amount of endotoxin added. Differences in potency correlated with differences in efficiency of LBP-dependent delivery to and extraction of endotoxin by CD14. Both membrane phospholipids and endotoxin are extracted by LBP/soluble CD14 (sCD14) treatment, but only endotoxin·sCD14 reacts with MD-2 and activates cells. These findings indicate that the proinflammatory potency of endotoxin may be regulated not only by the intrinsic structural properties of endotoxin but also by its association with neighboring mols. in the outer membrane.
CC 15-10 (Immunochemistry)
Section cross-reference(s): 10
ST membrane bleb **Neisseria** CD14 LBP **protein** monocyte activation; TLR4 receptor **MD2 protein** endothelium activation membrane bleb **Neisseria**
IT Human
(LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT CD14 (antigen)
RL: ANT (Analyte); ANST (Analytical study)
(LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT **Proteins**
RL: ANT (Analyte); ANST (Analytical study)
(**MD-2**; LBP/CD14- and TLR4/**MD-2** -dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Proteins
RL: ANT (Analyte); ANST (Analytical study)
(Op (opacity protein); of membrane blebs of group B **Neisseria** meningitidis)
IT Receptors
RL: ANT (Analyte); ANST (Analytical study)
(TLR-4 (Toll-like receptor-4); LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Monocyte
(activation; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)

IT Proteins
RL: ANT (Analyte); ANST (Analytical study)
(cationic; of membrane blebs of group B **Neisseria** meningitidis)
IT Blood vessel
(endothelium; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT **Neisseria** meningitidis
(group B; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Lipopolysaccharides
RL: ANT (Analyte); ANST (Analytical study)
(lipooligosaccharides; of membrane blebs of group B **Neisseria** meningitidis)
IT **Proteins**
RL: ANT (Analyte); ANST (Analytical study)
(lipopolysaccharide-**binding**; LBP/CD14- and TLR4/**MD**-**2**-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Cell activation
(monocyte; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Fatty acids, analysis
Phospholipids, analysis
Porins
RL: ANT (Analyte); ANST (Analytical study)
(of membrane blebs of group B **Neisseria** meningitidis)
IT Endothelium
(vascular; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
IT Organelle
(vesicle; LBP/CD14- and TLR4/MD-2-dependent activation of endothelial cells and monocytes by membrane blebs of group B **Neisseria** meningitidis)
REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 4 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 2005:489362 CAPLUS
DOCUMENT NUMBER: 143:95745
TITLE: Monomeric **endotoxin**:protein **complexes** are essential for TLR4-dependent cell activation
AUTHOR(S): Gioannini, T. L.; Teghanemt, A.; Zhang, De S.; Levis, E. N.; Weiss, J. P.
CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa and the Veterans' Administration Medical Center, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 09 Jun 2005
AB Potent TLR4-dependent cell activation by Gram-neg. bacterial endotoxin

depends on sequential endotoxin-protein and protein-protein interactions with LBP, CD14, MD-2 and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single endotoxin mols. from purified endotoxin aggregates (Eagg) or the bacterial outer membrane and form monomeric endotoxin:CD14 complexes that are the preferred presentation of endotoxin for transfer to MD-2. Endotoxin in endotoxin:CD14 is readily transferred to MD-2, again in an albumin-dependent manner, to form monomeric endotoxin:MD-2 complex. This monomeric endotoxin:protein complex (endotoxin:MD-2) activates TLR4 at picomolar concns., independently of albumin, and is, therefore, the apparent ligand in endotoxin-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of meningococcal endotoxin (LOS) react similarly with LBP, CD14, and MD-2 to form endotoxin:MD-2 complexes. However, tetra- and penta-acylated LOS:MD-2 complexes are less potent TLR4 agonists than hexa-acylated LOS:MD-2. This is mirrored in the reduced activity of tetra-, penta- vs. hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14, MD-2, and TLR4. Therefore, changes in agonist potency of under-acylated meningococcal LOS are determined by differences in properties of monomeric endotoxin:MD-2.

CC 15-10 (Immunochemistry)

ST **endotoxin** protein **complex** TLR4 receptor cell activation

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; monomeric **endotoxin**:**protein complexes** are essential for TLR4 receptor-dependent cell activation)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Blood vessel
(endothelium; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Toxins
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**endotoxins**; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Glycolipids
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(lipooligosaccharides; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Cell activation
(monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serum; monomeric **endotoxin**:protein **complexes** are essential for TLR4 receptor-dependent cell activation)

IT Endothelium
(vascular; monomeric **endotoxin**:protein **complexes**

are essential for TLR4 receptor-dependent cell activation)
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 5 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 2004:292853 CAPLUS
DOCUMENT NUMBER: 140:401625
TITLE: Isolation of an **endotoxin**-MD-2 **complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations
AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191 CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 09 Apr 2004
AB Host proinflammatory responses to minute amts. of endotoxins derived from many Gram-neg. bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and MD-2. Optimal sensitivity to endotoxin requires an ordered series of endotoxin-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of endotoxin aggregates to soluble CD14 (sCD14) to form monomeric endotoxin-sCD14 complexes. Subsequent interactions of endotoxin-sCD14 with TLR4 and/or MD-2 have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive endotoxin-MD-2 complex generated by treatment of endotoxin-sCD14 with recombinant MD-2. Efficient generation of this complex occurred at picomolar concns. of endotoxin and nanogram per mL doses of MD-2 and required presentation of endotoxin to MD-2 as a monomeric endotoxin-CD14 complex. TLR4-dependent delivery of endotoxin to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of endotoxin occurred with the purified endotoxin-MD-2 complex, but not with purified endotoxin aggregates with or without LBP and/or sCD14. The presence of excess MD-2 inhibited delivery of endotoxin-MD-2 to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of endotoxin occurs by sequential interaction and transfer of endotoxin to LBP, CD14, and MD-2 and simultaneous engagement of endotoxin and TLR4 by MD-2.
CC 4-5 (Toxicology)
ST **endotoxin** MD2 **complex** CD14 TLR4 LBP
IT Animal cell line
(HEK; isolation of an **endotoxin**-MD-2 **complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LPS-LBP (lipopolysaccharide-containing lipopolysaccharide-**binding protein**); isolation of an **endotoxin-MD-2 complex** that produces Toll-like receptor 4-dependent cell activation at picomolar concns. in HEK cells)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes** with **endotoxin**
; isolation of an **endotoxin**-**MD-2**
complex that produces Toll-like receptor 4-dependent cell
activation at picomolar concns. in HEK cells)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); isolation of an **endotoxin**-MD-2
complex that produces Toll-like receptor 4-dependent cell
activation at picomolar concns. in HEK cells)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**complexes** with **endotoxin**; isolation of an
endotoxin-MD-2 **complex** that produces Toll-like
receptor 4-dependent cell activation at picomolar concns. in HEK cells)
IT Toxins
RL: ADV (Adverse effect, including toxicity); BSU (Biological study,
unclassified); BIOL (Biological study)
(**endotoxins**, **complexes** with CD14 and MD-2;
isolation of an **endotoxin**-MD-2 **complex** that
produces Toll-like receptor 4-dependent cell activation at picomolar
concns. in HEK cells)
IT Human
(isolation of an **endotoxin**-MD-2 **complex** that
produces Toll-like receptor 4-dependent cell activation at picomolar
concns. in HEK cells)
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 6 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 10
ACCESSION NUMBER: 2004:1021053 CAPLUS
DOCUMENT NUMBER: 142:73002
TITLE: Potential Role of **Endotoxin** as a
Proinflammatory Mediator of Atherosclerosis
AUTHOR(S): Stoll, Lynn L.; Denning, Gerene M.; Weintraub, Neal L.
CORPORATE SOURCE: Department of Internal Medicine, Divisions of
Cardiovascular Diseases and Infectious Diseases,
University of Iowa and The VA Medical Center, Iowa
City, IA, USA
SOURCE: Arteriosclerosis, Thrombosis, and Vascular Biology
(2004), 24(12), 2227-2236
CODEN: ATVBFA; ISSN: 1079-5642
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
ED Entered STN: 29 Nov 2004
AB A review. Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Although a variety of inflammatory markers (ie, C-reactive protein) have been associated with atherosclerosis and its consequences, it is important to identify principal mediators of the inflammatory responses. One potentially important source of vascular inflammation in atherosclerosis is bacterial endotoxin. Mutations in Toll-like receptor 4 (TLR-4), an integral component of the endotoxin signaling complex, are fairly common in the Caucasian population and have recently been associated with reduced incidence of atherosclerosis and other cardiovascular diseases in some studies. Moreover, epidemiol. studies suggest that endotoxemia at levels as low as 50 pg/mL constitutes a strong risk factor for the development of atherosclerosis. Endotoxin concns. in this range may be produced by a variety of common subclin. Gram-neg.

infections. In this article, we outline the main elements of the endotoxin signaling receptor complex that initiates proinflammatory signaling (lipopolysaccharide binding protein [LBP], CD14, TLR-4, and MD-2) and discuss how changes in expression of these mols. may affect proatherogenic responses in the vessel wall. We also describe some of the proinflammatory effects of endotoxin that may be relevant to atherosclerosis, and discuss how serum lipoproteins, especially high-d. lipoprotein, may modulate endotoxin-induced inflammatory responses. Further, we discuss recent findings suggesting that the lipid-lowering statins may have an addnl. protective role in blocking at least some of these proinflammatory signaling pathways. Finally, we discuss species diversity with regard to endotoxin signaling that should be considered when extrapolating exptl. data from animal models to humans.

CC 15-0 (Immunochemistry)
Section cross-reference(s): 14

ST review **endotoxin** atherosclerosis signaling CD14 lipopolysaccharide **binding** protein

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LPS-LBP (lipopolysaccharide-containing lipopolysaccharide-**binding** protein); **endotoxin** as a proinflammatory mediator of atherosclerosis)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; **endotoxin** as a proinflammatory mediator of atherosclerosis)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); **endotoxin** as a proinflammatory mediator of atherosclerosis)

IT Human
Signal transduction, biological
(**endotoxin** as a proinflammatory mediator of atherosclerosis)

IT CD14 (antigen)
High-density lipoproteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxin** as a proinflammatory mediator of atherosclerosis)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; **endotoxin** as a proinflammatory mediator of atherosclerosis)

REFERENCE COUNT: 175 THERE ARE 175 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 7 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 11

ACCESSION NUMBER: 2004:1074780 CAPLUS
DOCUMENT NUMBER: 142:238068
TITLE: **Endotoxin** recognition and signal transduction by the TLR4/MD2-**complex**
AUTHOR(S): Fitzgerald, Katherine A.; Rowe, Daniel C.; Golenbock, Douglas T.
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, 01605, USA
SOURCE: Microbes and Infection (2004), 6(15), 1361-1367
CODEN: MCINFS; ISSN: 1286-4579
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

ED Entered STN: 16 Dec 2004
AB A review. Bacterial lipopolysaccharides are recognized in mammals by a receptor complex composed of CD14, Toll-like receptor (TLR)-4, and MD-2. Transduction of signaling is achieved following the recruitment of a combination of four Toll-interleukin-1 resistance (TIR)-domain-containing adapter mols., which provide a structural platform enabling the recruitment and activation of downstream effectors essential for pathway-specific transcription factor activation and inflammatory gene expression.
CC 15-0 (Immunochemistry)
Section cross-reference(s): 14
ST review **endotoxin** lipopolysaccharide recognition signaling TLR4 MD2 **complex**
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; bacterial lipopolysaccharide/ **endotoxin** recognition and signal transduction by TLR4/ **MD2 complex**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); bacterial lipopolysaccharide/ **endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by CD14/TLR4/MD2 **complex**)
IT Inflammation
Signal transduction, biological
(bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; bacterial lipopolysaccharide/**endotoxin** recognition and signal transduction by TLR4/MD2 **complex**)
REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 8 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 14
ACCESSION NUMBER: 2004:70743 CAPLUS
DOCUMENT NUMBER: 140:355309
TITLE: **Endotoxin** recognition molecules, Toll-like receptor 4-MD-2
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: The Institute of Medical Science, Department of Microbiology and Immunology, Division of Infectious Genetics, The University of Tokyo, 4-6-1 Shirokanedai, Tokyo, 108-8639, Japan
SOURCE: Seminars in Immunology (2004), 16(1), 11-16
CODEN: SEIME2; ISSN: 1044-5323
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
ED Entered STN: 29 Jan 2004
AB A review. Toll-like receptors (TLRs) are innate pathogen recognition mols. for microbial products. Lipopolysaccharide (LPS), a membrane constituent of Gram-neg. bacteria, is one of the most potent microbial products. LPS is recognized by TLR4 and MD-2. TLR4 is a transmembrane protein, the extracellular domain of which is composed of a protein motif called leucine-rich repeats (LRR). MD-2 is an extracellular mol. that is

associated with the extracellular LRR of TLR4. MD-2 has a role in cell surface expression of TLR4 and interaction with LPS. TLR4-MD-2 contributes to containment of infections by Gram-neg. bacteria by activating immune responses.

CC 15-0 (Immunochemistry)

ST review **endotoxin** TLR4 receptor **complex MD2 protein**

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes**, with TLR-4; in immune recognition of bacterial **endotoxin**)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complexes**, with MD-2; in immune recognition of bacterial **endotoxin**)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; TLR-4 receptor/**MD-2** accessory **protein** in immune recognition of)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; TLR-4 receptor/**MD-2** accessory **protein** in immune recognition of)

IT Immunity
(innate; TLR-4 receptor/**MD-2** accessory **protein** in recognition of bacterial **endotoxin**)

REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 9 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 21

ACCESSION NUMBER: 2001:491171 CAPLUS

DOCUMENT NUMBER: 136:149763

TITLE: Molecular genetic analysis of an **endotoxin** nonresponder mutant cell line: a point mutation in a conserved region of MD-2 abolishes **endotoxin** -induced signaling

AUTHOR(S): Schromm, Andra B.; Lien, Egil; Henneke, Philipp; Chow, Jesse C.; Yoshimura, Atsutoshi; Heine, Holger; Latz, Eicke; Monks, Brian G.; Schwartz, David A.; Miyake, Kensuke; Golenbock, Douglas T.

CORPORATE SOURCE: Evans Biomedical Research Center, Boston University School of Medicine, Boston, MA, 02118, USA

SOURCE: Journal of Experimental Medicine (2001), 194(1), 79-88
CODEN: JEMEAV; ISSN: 0022-1007

PUBLISHER: Rockefeller University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

ED Entered STN: 08 Jul 2001

AB Somatic cell mutagenesis is a powerful tool for characterizing receptor systems. We reported previously two complementation groups of mutant cell lines derived from CD14-transfected Chinese hamster ovary-K1 fibroblasts defective in responses to bacterial endotoxin. Both classes of mutants expressed a normal gene product for Toll-like receptor (TLR)4, and fully responded to stimulation by tumor necrosis factor (TNF)-α or interleukin (IL)-1β. We identified the lesion in one of the complementation groups in the gene for MD-2, a putative TLR4 coreceptor. The nonresponder phenotype of this mutant was reversed by transfection with MD-2. Cloning of MD-2 from the nonresponder cell line revealed a point mutation in a highly conserved region resulting in a C95Y amino acid exchange. Both forms of MD-2 colocalized with TLR4 on the cell surface

after transfection, but only the wild-type cDNA reverted the lipopolysaccharide (LPS) nonresponder phenotype. Furthermore, soluble MD-2, but not soluble MD-2C95Y, functioned to enable LPS responses in cells that expressed TLR4. Thus, MD-2 is a required component of the LPS signaling complex and can function as a soluble receptor for cells that do not otherwise express it. We hypothesize that MD-2 conformationally affects the extracellular domain of TLR4, perhaps resulting in a change in affinity for LPS or functioning as a portion of the true ligand for TLR4.

CC 15-10 (Immunochemistry)

ST MD2 Toll like receptor **endotoxin** signaling; lipopolysaccharide signaling MD2 TLR4

IT Signal transduction, biological
(LPS signaling, MD-2 is required component of; point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(LPS, MD-2 is required component of LPS signaling **complex**; point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

IT **Proteins**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**MD-2**, TLR4 coreceptor; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), MD-2 colocalized with; point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

IT **Protein** motifs
(conserved region, of **MD-2**, C95Y substitution in; point mutation in conserved region of **MD-2** abolishes **endotoxin**-induced signaling)

IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**, LPS; point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

IT Gram-negative bacteria
(point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

IT Mutation
(point; point mutation in conserved region of MD-2 abolishes **endotoxin**-induced signaling)

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 10 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2006:303353 CAPLUS

DOCUMENT NUMBER: 144:329754

TITLE: R-form LPS, the master key to the activation of TLR4/MD-2-positive cells

AUTHOR(S): Huber, Michael; Kalis, Christoph; Keck, Simone; Jiang, Zhengfan; Georgel, Philippe; Du, Xin; Shamel, Louis; Sovath, Sosathya; Mudd, Suzanne; Beutler, Bruce; Galanos, Chris; Freudenberg, Marina A.

CORPORATE SOURCE: Molecular Immunology, Institute for Biology III, Albert-Ludwigs University Freiburg, Freiburg, Germany

SOURCE: European Journal of Immunology (2006), 36(3), 701-711

CODEN: EJIMAF; ISSN: 0014-2980
PUBLISHER: Wiley-VCH Verlag GmbH & Co. KGaA
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 03 Apr 2006
AB Lipopolysaccharide (endotoxin, LPS) is a major recognition marker for the detection of gram-neg. bacteria by the host and a powerful initiator of the inflammatory response to infection. Using S- and R-form LPS from wild-type and R-mutants of Salmonella and E. coli, we show that R-form LPS readily activates mouse cells expressing the signaling receptor Toll-like receptor 4/myeloid differentiation protein 2 (TLR4/MD-2), while the S-form requires further the help of the LPS-binding proteins CD14 and LBP, which limits its activating capacity. Therefore, the R-form LPS under physiol. conditions recruits a larger spectrum of cells in endotoxic reactions than S-form LPS. We also show that soluble CD14 at high concns. enables CD14-neg. cells to respond to S-form LPS. The presented in vitro data are corroborated by an in vivo study measuring TNF-α levels in response to injection of R- and S-form LPS in mice. Since the R-form LPS constitutes ubiquitously part of the total LPS present in all wild-type bacteria, its contribution to the innate immune response and pathophysiol. of infection is much higher than anticipated during the last half century.
CC 15-10 (Immunochemistry)
IT Toxins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**endotoxins**; R-form lipopolysaccharide activates TLR4/MD-2-pos. cells)
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipopolysaccharide-**binding**; R-form lipopolysaccharide activates TLR4/**MD-2**-pos. cells)
REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 11 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1016749 CAPLUS
DOCUMENT NUMBER: 143:304609
TITLE: MD-2 Mediates the Ability of Tetra-Acylated and Penta-Acylated Lipopolysaccharides to Antagonize **Escherichia** coli Lipopolysaccharide at the TLR4 Signaling **Complex**
AUTHOR(S): Coats, Stephen R.; Pham, Thu-Thao T.; Bainbridge, Brian W.; Reife, Robert A.; Darveau, Richard P.
CORPORATE SOURCE: Department of Periodontics, University of Washington School of Dentistry, Seattle, WA, 98195, USA
SOURCE: Journal of Immunology (2005), 175(7), 4490-4498
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 21 Sep 2005
AB The authors have demonstrated previously that tetra-acylated LPS derived from the oral bacterium, Porphyromonas gingivalis, and penta-acylated msbB LPS derived from a mutant strain of Escherichia coli can antagonize the ability of canonical hexa-acylated E. coli LPS to signal through the TLR4 signaling complex in human endothelial cells. Activation of the TLR4 signaling complex requires the coordinated function of LPS binding protein (LBP), CD14, MD-2, and TLR4. To elucidate the specific mol. components that mediate antagonism, the authors developed a recombinant human TLR4 signaling complex that displayed efficient LPS-dependent antagonism of E. coli LPS in HEK293 cells. Notably, changes in the expression levels of

TLR4 in HEK293 cells modulated the efficiency of antagonism by P. gingivalis LPS. Both soluble (s) CD14 and membrane (m) CD14 supported efficient P. gingivalis LPS-dependent and msbB LPS-dependent antagonism of E. coli LPS in the recombinant TLR4 system. When cells expressing TLR4, MD-2, and mCD14 were exposed to LPS in the absence of serum-derived LBP, efficient LPS-dependent antagonism of E. coli LPS was still observed indicating that LPS-dependent antagonism occurs downstream of LBP. Expts. using immunoppts. of sCD14 or sMD-2 that had been pre-exposed to agonist and antagonist indicated that LPS-dependent antagonism occurs partially at sCD14 and potently at sMD-2. This study provides novel evidence that expression levels of TLR4 can modulate the efficiency of LPS-dependent antagonism. However, MD-2 represents the principal mol. component that tetra-acylated P. gingivalis LPS and penta-acylated msbB LPS use to antagonize hexa-acylated E. coli LPS at the TLR4 signaling complex.

CC 15-10 (Immunochemistry)

ST **MD2 protein** lipopolysaccharide structure TLR4 receptor signaling

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**Escherichia** coli; MD-2 mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

IT **Escherichia** coli
Human
Porphyromonas gingivalis
Signal transduction, biological
(MD-2 mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MD-2 mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; **MD-2** mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); MD-2 mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(penta-acylated and tetra-acylated; MD-2 mediates tetra-acylated and penta-acylated lipopolysaccharide antagonism of Toll-like receptor-4 signaling response to **Escherichia** coli lipopolysaccharide)

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 12 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:862915 CAPLUS
TITLE: Lipopolysaccharide recognition **protein**, **MD-2**, facilitates cellular uptake of E. coli-derived plasmid DNA in synovium
AUTHOR(S): Kolka, Jacquelyn A.; Vreede, Andrew P.; Roessler, Blake J.

CORPORATE SOURCE: Division of Rheumatology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, 48109-0688, USA
SOURCE: Journal of Gene Medicine (2005), 7(7), 956-964
CODEN: JGMEFG; ISSN: 1099-498X
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 23 Aug 2005

AB Several cell types are susceptible to transfection in vivo using naked plasmid DNA. The mechanisms involved in mediating in vivo transfection are incompletely known, but evidence suggests that receptor-mediated endocytosis is important for specific types of cells. In this study the authors tested the hypothesis that residual Escherichia coli lipopolysaccharide (LPS) forms a non-covalent complex with expression plasmid DNA, and host-cell-derived soluble LPS-binding proteins bind to the DNA-LPS complexes to facilitate receptor-mediated endocytosis. Cells from the murine synovial lining were used as an in vivo model system and in vivo luciferase imaging was used to quantify levels of transgene expression. Using a series of gene-deleted mice, the roles of LPS recognition complex proteins, lipopolysaccharide-binding protein (LBP), CD14 and MD-2, in the process of in vivo transfection were determined Luciferase expression assays revealed that mice lacking LBP or CD14 had increased luciferase expression, while mice deleted of MD-2 had significant redns. in luciferase expression. Gene deletion of hyaluronic acid binding protein CD44 was used as a control and had no statistically significant effect on transgene expression in vivo. In muscle tissue, where neither cell surface nor soluble MD-2 is expressed, no MD-2 dependence of plasmid transfection was identified, suggesting the role of MD-2 is tissue or cell type specific. Addnl., depleting mice of macrophages showed that luciferase expression is occurring within fibroblast-like synoviocytes. The authors' data support a phys. association between LPS and E. coli-derived plasmid DNA, and that in vivo transfection of fibroblast-like synoviocytes is dependent on the soluble form of the LPS-binding protein MD-2.

CC 3-4 (Biochemical Genetics)
Section cross-reference(s): 15

IT **Escherichia** coli
Macrophage
Plasmids
Synovial membrane
Transformation, genetic
(lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)

IT CD14 (antigen)
Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipopolysaccharide-**binding**; lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)

IT Endocytosis
(receptor-mediated; lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E.

coli-derived plasmid DNA in synovium and involved mechanisms)
IT Muscle
(skeletal; lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)
IT Synovial membrane
(synoviocyte; lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)
IT Biological transport
(uptake; lipopolysaccharide recognition **protein MD-2** facilitation of cellular uptake of E. coli-derived plasmid DNA in synovium and involved mechanisms)
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 13 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:1089125 CAPLUS
DOCUMENT NUMBER: 142:175247
TITLE: Protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions
AUTHOR(S): Asai, Yasuyuki; Takaori, Kyoichi; Yamamoto, Tsuyoshi; Ogawa, Tomohiko
CORPORATE SOURCE: Department of Oral Microbiology, Asahi University School of Dentistry, Mizuho, Gifu, 501-0296, Japan
SOURCE: FEMS Immunology and Medical Microbiology (2005), 43(1), 91-98
CODEN: FIMIEV; ISSN: 0928-8244
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 20 Dec 2004
AB The protein-bound polysaccharide isolated from basidiomycetes (PSK) is a biol. response modifier capable of exhibiting various biol. activities, such as antitumor and antimicrobial effects. In the present study, the authors found that PSK suppressed interleukin (IL)-6 production in murine peritoneal macrophages stimulated with endotoxic lipopolysaccharide (LPS) and its synthetic lipid A (compound 506). Nitric oxide production and p38 mitogen-associated protein kinase phosphorylation induced in a murine macrophage cell line, J774-A1, by LPS and compound 506 were also inhibited by PSK. Further, PSK distinctly suppressed nuclear factor-κB activation in Ba/F3 cells expressing mouse Toll-like receptor 4 and MD-2, following stimulation with LPS and compound 506, however, not with Taxol. These PSK-induced inhibitory activities were caused by inhibition of the phys. assocns. of LPS with LPS-binding protein (LBP) and CD14. PSK also protected mice from LPS-induced lethality, presumably by down-regulating IL-6 and tumor necrosis factor-α concns. in serum. These findings indicate that PSK, which also has an ability to regulate LBP/CD14 functions, may be useful for clin. control of endotoxic sepsis.
CC 15-10 (Immunochemistry)
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; **protein-bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding protein** and CD14 functions)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(NF-κB (nuclear factor of κ light chain gene enhancer in B-cells); protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipopolysaccharide-**binding**; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Peritoneum
(macrophage; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Macrophage
(peritoneal; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Basidiomycota
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT CD14 (antigen)
Interleukin 6
Tumor necrosis factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT Polysaccharides, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound**; protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

IT 10102-43-9, Nitric oxide, biological studies 165245-96-5, p38 MAP kinase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein-**bound** polysaccharide isolated from basidiomycetes inhibits **endotoxin**-induced activation by blocking lipopolysaccharide-**binding** protein and CD14 functions)

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 14 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:102155 CAPLUS
DOCUMENT NUMBER: 143:226504
TITLE: Roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells
AUTHOR(S): Xiong, Jianqiong; Zhu, Peifang; Wang, Zhengguo; Jiang, Jianxin

CORPORATE SOURCE: Daping Hospital, Third Military Medical University, Chongqing, 400042, Peop. Rep. China
SOURCE: Di-San Junyi Daxue Xuebao (2004), 26(24), 2235-2238
CODEN: DYXUE8; ISSN: 1000-5404
PUBLISHER: Di-San Junyi Daxue Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
ED Entered STN: 07 Feb 2005
AB To investigate the expression of myeloid differentiation protein-2 (MD-2) in the human endothelial cells and its roles in the binding of lipopolysaccharide (LPS) to endothelial cells, based on cultured human umbilical vein endothelial cells (HUVECs), the expression of MD-2 and the effects of LPS on the expression of MD-2 were analyzed by RT-PCR and Western blot. The roles of blood serum, Toll-like receptor 4 (TLR4), and MD-2 in LPS binding to endothelial cells were analyzed by flow cytometry. The results showed that MD-2 was expressed in human endothelial cells and LPS could upregulate the expression of MD-2 in time- and dose-dependent manners. Blood serum could obviously promote LPS binding to endothelial cells. Anti-TLR4 and anti-MD-2 monoclonal antibodies significantly inhibited LPS binding to cells in a dose-dependent manner. MD-2 may play an important role in the binding of LPS to endothelial cells.
CC 13-3 (Mammalian Biochemistry)
ST myeloid differentiation protein 2 **endotoxin** endothelium
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; roles of myeloid differentiation **protein**-2 in **binding** of lipopolysaccharide to human endothelial cells)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells)
IT Human
(roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells)
IT Gene expression
Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells)
IT Endothelium
(umbilical venous; roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells)
IT Vein
(umbilical, endothelium; roles of myeloid differentiation protein-2 in **binding** of lipopolysaccharide to human endothelial cells)

L60 ANSWER 15 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:501450 CAPLUS
DOCUMENT NUMBER: 141:205606
TITLE: Lipid A antagonist, lipid IVa, is distinct from lipid A in interaction with Toll-like receptor 4 (TLR4)-MD-2 and ligand-induced TLR4 oligomerization
AUTHOR(S): Saitoh, Shin-ichiroh; Akashi, Sachiko; Yamada, Takenao; Tanimura, Natsuko; Kobayashi, Makiko; Konno, Kazunori; Matsumoto, Fumi; Fukase, Koichi; Kusumoto, Shoichi; Nagai, Yoshinori; Kusumoto, Yutaka; Kosugi, Atsushi; Miyake, Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, Institute of Medical

Science, University of Tokyo, Tokyo, Japan
SOURCE: International Immunology (2004), 16(7), 961-969
CODEN: INIMEN; ISSN: 0953-8178
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 22 Jun 2004
AB Toll-like receptor 4 (TLR4) and MD-2 recognizes lipid A, the active moiety of microbial lipopolysaccharide (LPS). Little is known about mechanisms for LPS recognition by TLR4-MD-2. Here the authors show ligand-induced TLR4 oligomerization, homotypic interaction of TLR4, which directly leads to TLR4 signaling. Since TLR4 oligomerization normally occurred in the absence of the cytoplasmic portion of TLR4, TLR4 oligomerization works upstream of TLR4 signaling. Lipid IVa, a lipid A precursor, is agonistic on mouse TLR4-MD-2 but turns antagonistic on chimeric mouse TLR4-human MD-2, demonstrating that the antagonistic activity of lipid IVa is determined by human MD-2. Binding studies with radioactive lipid A and lipid IVa revealed that lipid IVa is similar to lipid A in dose-dependent and saturable binding to mouse TLR4-human MD-2. Lipid IVa, however, did not induce TLR4 oligomerization, and inhibited lipid A-dependent oligomerization of mouse TLR4-human MD-2. Thus, lipid IVa binds mouse TLR4-human MD-2 but does not trigger TLR4 oligomerization. Binding study further revealed that the antagonistic activity of lipid IVa correlates with augmented maximal binding to mouse TLR4-human MD-2, which was .apprx.2-fold higher than lipid A. Taken together, lipid A antagonist lipid IVa is distinct from lipid A in binding to TLR4-MD-2 and in subsequent triggering of TLR4 oligomerization. Given that the antagonistic activity of lipid IVa is determined by MD-2, MD-2 has an important role in a link between ligand interaction and TLR4 oligomerization.
CC 15-10 (Immunochemistry)
ST lipopolysaccharide TLR4 receptor oligomerization signaling; lipid A antagonist TLR4 receptor **MD2 protein** signaling
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**Escherichia** coli; structural requirements for ligand-induced oligomerization and signaling by Toll-like receptor-4)
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; is required for ligand-induced oligomerization of Toll-like receptor-4)
IT **Protein** motifs
(glycosylation site; of **MD-2** is required for ligand-induced oligomerization of Toll-like receptor-4)
IT 91841-27-9, Lipid IVa
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interaction with TLR-4 receptor-MD-2 **complex**)
REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 16 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:683145 CAPLUS
DOCUMENT NUMBER: 142:296120
TITLE: Molecular mechanism of **endotoxin** (LPS) recognition
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: Institute of Medical Science, University of Tokyo, Japan
SOURCE: Endotokishin Kenkyu (2003), 6, 23-30
CODEN: EKNEBO
PUBLISHER: Igaku Tosho Shuppan K.K.

DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
ED Entered STN: 23 Aug 2004
AB A review. The topics discussed are (1) processing of lipopolysaccharide (LPS); (2) Toll-like receptor 4 (TLR4) in the recognition of LPS; (3) MD-2 binding to TLR4; (4) MD-2 required for cell surface expression of TLR4; (5) role of MD-2 in TLR4 recognition of LPS; and (6) RP105-MD-1 complex in B cell recognition of LPS.
CC 15-0 (Immunochemistry)
ST review Toll like receptor TLR4 MD2 **endotoxin** lipopolysaccharide
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complexes** with TLR4; Toll-like receptor 4-**MD-2 complex** in recognition of **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complexes** with MD-2; Toll-like receptor 4-MD-2 **complex** in recognition of **endotoxin**
)
IT Molecular association
Molecular recognition
(Toll-like receptor 4-MD-2 **complex** in recognition of **endotoxin**)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; Toll-like receptor 4-MD-2 **complex** in recognition of **endotoxin**)
IT Immunity
(innate; Toll-like receptor 4-MD-2 **complex** in recognition of **endotoxin**)

L60 ANSWER 17 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:268371 CAPLUS
DOCUMENT NUMBER: 136:400270
TITLE: Identification of LPS-**Binding** Peptide Fragment of **MD-2**, a Toll-Receptor Accessory **Protein**
AUTHOR(S): Mancek, Mateja; Pristovsek, Primoz; Jerala, Roman
CORPORATE SOURCE: National Institute of Chemistry, Ljubljana, SI-1000, Slovenia
SOURCE: Biochemical and Biophysical Research Communications (2002), 292(4), 880-885
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 10 Apr 2002
AB Members of the toll-like receptor family are crucial in recognition of microbial pathogens as part of innate immune response. MD-2, an accessory protein to TLR4, present on the extracellular side of the membrane is needed to initiate the signal transduction. We have identified a 15 amino acid region of human MD-2 that contains several features of other lipopolysaccharide (LPS) binding proteins and peptides. In vitro LPS neutralization by this peptide was observed and confirmed by 2D transferred NOESY NMR expts. NMR expts. have also shown binding of the MD-2 peptide to lipoteichoic acid (LTA) but not to peptidoglycan. Furthermore this peptide inhibited growth of gram-neg. and to a lower extent of some gram-pos. bacteria. Our results indicate that this region of MD-2 might be responsible for binding of LPS and confirms the role of MD-2 as an

accessory protein in LPS signaling bestowing the Toll receptors their specificity.
CC 15-5 (Immunochemistry)
ST lipopolysaccharide **binding** peptide MD2 Toll receptor
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**; identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Toll; identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**)
IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial, peptide-**binding**; identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**)
IT Human
(identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**)
IT Firmicutes
Gram-negative bacteria
(identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**, and inhibition of)
IT 428867-19-0P
RL: BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(**MD-2**; identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**)
IT 9041-38-7D, Teichoic acid, lipo-
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lipoteichoic acid; identification of LPS-**binding** peptide fragment of **MD-2**, a Toll-receptor accessory **protein**, and **binding** to)
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 18 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:725654 CAPLUS
DOCUMENT NUMBER: 137:230827
TITLE: Interaction of RP 105/MD-1 **complex** and TLR 4 in **Gram-negative bacteria** recognition of B cell
AUTHOR(S): Kikuchi, Takane; Miyake, Kensuke
CORPORATE SOURCE: The Inst. Med. Sci., The Univ. Tokyo, Japan
SOURCE: Ensho to Men'eki (2002), 10(5), 567-573
CODEN: ENMEFA; ISSN: 0918-8371
PUBLISHER: Sentan Igakusha
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
ED Entered STN: 25 Sep 2002
AB A review on the discovery of Toll-like receptors (TLR), ligands of TLR family, roles of CD14 and TLR4 in the recognition of lipopolysaccharides (LPS), LPS recognition by TLR4/MD-2, and possible involvement of RP105/MD-1 complex in the regulation of LPS signaling in B cells.
CC 15-0 (Immunochemistry)
IT **Proteins**

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(MD-1, **MD-2**, and RP105; mol. mechanism of lipopolysaccharide recognition by B cell)

L60 ANSWER 19 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:588215 CAPLUS
DOCUMENT NUMBER: 138:70580
TITLE: TLR4-MD2 signaling pathway induced by **endotoxin**
AUTHOR(S): Li, Yongwang; Ma, Li; Mao, Baoling; Qian, Guisheng
CORPORATE SOURCE: Institute of Respiratory Disease, Xinqiao Hospital, the Third Military Medical University, Chungking, 400037, Peop. Rep. China
SOURCE: Zhongguo Yaolixue Tongbao (2002), 18(2), 121-125
CODEN: ZYTOE8; ISSN: 1001-1978
PUBLISHER: Anhui Yike Daxue Linchuan Yaoli Yanjiuso
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Chinese
ED Entered STN: 08 Aug 2002
AB A review with 24 refs. on TLR4-MD2 (TLR4 = toll-like receptor-4) signaling pathway induced by endotoxin with subdivision headings: (1) survey on TLRs; (2) role of TLR4 and its accessory protein MD2 in signaling pathway; (3) basic composition of lipopolysaccharide (LPS) signaling pathway mediated by TLR4-MD2; Biol. significance of TLR4-MD2 signaling pathway deficiency; (5) expression and role of TLR4-MD2 in different tissues; and (6) conclusions.
CC 14-0 (Mammalian Pathological Biochemistry)
ST review TLR4 MD2 signaling pathway **endotoxin** inflammation
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2**, **complex** with TLR-4; TLR4-**MD2** signaling pathway induced by **endotoxin**)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(NF-κB (nuclear factor of κ light chain gene enhancer in B-cells); TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4), **complex** with MD-2; TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Inflammation
Signal transduction, biological
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Lipopolysaccharides
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR4- MD2 signaling pathway induced by **endotoxin**)
IT Toxins
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(**endotoxins**; TLR4- MD2 signaling pathway induced by **endotoxin**)

L60 ANSWER 20 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:817878 CAPLUS
DOCUMENT NUMBER: 136:117227
TITLE: MD-2 **binds** to bacterial lipopolysaccharide
AUTHOR(S): Viriyakosol, Suganya; Tobias, Peter S.; Kitchens, Richard L.; Kirkland, Theo N.

CORPORATE SOURCE: Veterans Administration San Diego Healthcare System and Department of Pathology and Medicine, University of California San Diego, San Diego, CA, 92161, USA
SOURCE: Journal of Biological Chemistry (2001), 276(41), 38044-38051
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 11 Nov 2001

AB The exact roles and abilities of the individual components of the lipopolysaccharide (LPS) receptor complex of proteins remain unclear. MD-2 is a mol. found in association with toll-like receptor 4 (TLR4). The authors produced recombinant human MD-2 to explore its LPS binding ability and role in the LPS receptor complex. MD-2 binds to highly purified rough LPS derived from Salmonella minnesota and Escherichia coli in 5 different assays; one assay yielded an apparent KD of 65 nM. MD-2 binding to LPS did not require LPS-binding proteins LBP and CD14; in fact LBP competed with MD-2 for LPS. MD-2 enhanced the biol. activity of LPS in TLR4-transfected Chinese hamster ovary cells but inhibited LPS activation of U373 astrocytoma cells and of monocytes in human whole blood. These data indicate that MD-2 is a genuine LPS-binding protein and strongly suggest that MD-2 could play a role in regulation of cellular activation by LPS depending on its local availability.

CC 15-8 (Immunochemistry)
ST MD2 **binding** bacterial lipopolysaccharide infection

IT **Escherichia** coli
Human
Molecular association
Salmonella minnesota
(MD-2 **binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT CD14 (antigen)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MD-2 **binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**MD-2; MD-2 binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TLR-4 (Toll-like receptor-4); MD-2 **binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT Infection
(bacterial; MD-2 **binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT Lipopolysaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(bacterial; MD-2 **binds** directly to bacterial lipopolysaccharide without assistance from either LBP or CD14 in cell response to microbial invasion)

IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(lipopolysaccharide-**binding**; **MD-2**
binds directly to bacterial lipopolysaccharide without
assistance from either LBP or CD14 in cell response to microbial
invasion)
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 21 OF 50 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:51060 CAPLUS
DOCUMENT NUMBER: 134:146371
TITLE: Toll-like receptors and associated MD molecules: roles for pathogen recognition in the immune system
AUTHOR(S): Miyake, Kensuke; Kimoto, Masao
CORPORATE SOURCE: Dep. Immunol., Saga Med. Sch., Japan
SOURCE: Saishin Igaku (2001), 56(1), 136-154
CODEN: SAIGAK; ISSN: 0370-8241
PUBLISHER: Saishin Igakusha
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
ED Entered STN: 19 Jan 2001
AB The Toll receptor in Drosophila has been implicated in recognition of fungal infection and activation of defense programs. Humans also have homologs of the Toll receptor, Toll-like receptors (TLR). TLRs have also been implicated in pathogen recognition. TLR2 recognizes Gram-pos. bacteria, mycoplasmas, and mycobacteria, whereas TLR4 recognizes lipopolysaccharide (LPS) from Gram-neg. bacteria. The authors have cloned the MD-2 mol. that is associated with the extracellular domain of TLR4, and shown that the LPS recognition/signaling via TLR4 is dependent on MD-2 association Expression of the TLR4-MD-2 complex on normal mouse macrophages and its LPS signaling were confirmed by the mAb specific for the TLR4/MD-2 complex. The authors also discovered another cell surface complex RP105/MD-1, which is expressed on B cells and delivers an activation signal, leading to potent B cell proliferation and resistance against B cell apoptosis. To understand a role of RP105/MD-1 in the immune system, the authors have made mice lacking RP105. B cells lacking RP105 showed hyporesponsiveness in LPS-induced proliferation and antibody formation. RP105/MD-1 therefore regulates the LPS signaling. In vitro studies suggested that RP105/MD-1 serves as MD-2 in helping TLR4 to recognize and signal LPS. Taken together with results of TLR4 and MD-2, LPS is recognized and signaled by a multi-mol. complex consisting of TLR4, MD-2, RP105, and MD-1. LPS induces a variety of responses in a variety of cells. Configuration of the complex may be different among cell types and might reflect a variety of responses induced by LPS.
CC 15-10 (Immunochemistry)
Section cross-reference(s): 3
ST Toll like receptor MD **protein complex** pathogen recognition immune; sequence **protein MD2** cDNA human
IT Cell activation
Cell proliferation
(B cell; cDNA sequence of human **MD-2**
protein and Toll-like receptors association with MD mols. in pathogen recognition in immune system)
IT **Proteins**, specific or class
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(MD-1; cDNA sequence of human **MD-2 protein**
and Toll-like receptors association with MD mols. in pathogen recognition in immune system)
IT **Proteins**, specific or class

RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(**MD-2**; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT **Proteins**, specific or class
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(RP105 **protein**; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT Receptors
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(TLR4 (Toll-like receptor 4); cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT Lipopolysaccharides
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Toll-like receptor recognition of; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT B cell (lymphocyte)
(activation; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT **Gram-negative bacteria**
(cDNA sequence of human **MD-2 protein** and Toll-like receptors and associated MD mols. in pathogen recognition in immune system)

IT B cell (lymphocyte)
Macrophage
Molecular association
Protein sequences
Signal transduction, biological
cDNA sequences
(cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT B cell (lymphocyte)
(proliferation; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT Apoptosis
(resistance to; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system in relation to)

IT 223245-59-8, **Protein** (human uterus clone OHP106)
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

IT 322482-53-1
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(nucleotide sequence; cDNA sequence of human **MD-2 protein** and Toll-like receptors association with MD mols. in pathogen recognition in immune system)

L60 ANSWER 22 OF 50 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2005593451 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16272300
TITLE: Pharmacological inhibition of **endotoxin** responses is achieved by targeting the TLR4 coreceptor, **MD-2**.
AUTHOR: Visintin Alberto; Halmen Kristen A; Latz Eicke; Monks Brian G; Golenbock Douglas T
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01655, USA.. alberto.visintin@umassmws.edu
CONTRACT NUMBER: AI 52455 (NIAID)
RO1 GM54060 (NIGMS)
RR14466 (NCRR)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2005 Nov 15) Vol. 175, No. 10, pp. 6465-72.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200601
ENTRY DATE: Entered STN: 8 Nov 2005
Last Updated on STN: 4 Jan 2006
Entered Medline: 3 Jan 2006

AB The detection of Gram-negative LPS depends upon the proper function of the TLR4-MD-2 receptor **complex** in immune cells. TLR4 is the signal transduction component of the LPS receptor, whereas **MD-2** is the **endotoxin-binding** unit. MD-2 appears to activate TLR4 when **bound** to TLR4 and ligated by LPS. Only the monomeric form of MD-2 was found to **bind** LPS and only monomeric MD-2 interacts with TLR4. Monomeric **MD-2** **binds** TLR4 with an apparent Kd of 12 nM; this **binding** avidity was unaltered in the presence of **endotoxin.** E5564, an LPS antagonist, appears to inhibit cellular activation by competitively preventing the **binding** of LPS to MD-2. Depletion of endogenous soluble MD-2 from human serum, with an immobilized TLR4 fusion protein, abrogated TLR4-mediated LPS responses. By determining the concentration of added-back MD-2 that restored normal LPS responsiveness, the concentration of MD-2 was estimated to be approximately 50 nM. Similarly, purified TLR4-Fc fusion protein, when added to the supernatants of TLR4-expressing cells in culture, inhibited the interaction of MD-2 with TLR4, thus preventing LPS stimulation. The ability to inhibit the effects of LPS as a result of the **binding** of TLR4-Fc or E5564 to **MD-2** highlights **MD-2** as the logical target for drug therapies designed to pharmacologically intervene against **endotoxin**-induced disease.

CT Cell Line
Humans
Kinetics
Lipid A: AA, analogs & derivatives
Lipid A: PD, pharmacology
Lipopolysaccharides: AI, antagonists & inhibitors

Lipopolysaccharides: ME, metabolism
*Lipopolysaccharides: TO, toxicity
Lymphocyte Antigen 96: BL, blood
Lymphocyte Antigen 96: CH, chemistry
*Lymphocyte Antigen 96: ME, metabolism
Protein Binding: DE, drug effects
Protein Structure, Tertiary
Recombinant Fusion Proteins: ME, metabolism
Recombinant Fusion Proteins: PD, pharmacology
Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, Non-P.H.S.
Signal Transduction
Solubility
Toll-Like Receptor 4: CH, chemistry
*Toll-Like Receptor 4: ME, metabolism

L60 ANSWER 23 OF 50 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2005505043 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16177114
TITLE: Molecular basis of reduced potency of underacylated endotoxins.
AUTHOR: Teghanemt Athmane; Zhang DeSheng; Levis Erika N; Weiss Jerrold P; Gioannini Theresa L
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA 52241, USA.
CONTRACT NUMBER: AI59372 (NIAID)
PO144642
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2005 Oct 1) Vol. 175, No. 7, pp. 4669-76.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200511
ENTRY DATE: Entered STN: 23 Sep 2005
Last Updated on STN: 15 Dec 2005
Entered Medline: 28 Nov 2005

AB Potent TLR4-dependent cell activation by gram-negative bacterial **endotoxins** depends on sequential **endotoxin**-protein and protein-protein interactions with LPS-**binding** protein, CD14, myeloid differentiation protein 2 (**MD-2**), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated endotoxins (i.e., tetra- or penta- vs hexa-acylated) is determined by post-CD14 interactions. To better define the molecular basis of the differences in agonist potency of endotoxins differing in fatty acid acylation, we compared endotoxins (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB meningococcal strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of endotoxin:protein and endotoxin:cell interactions, the endotoxins were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric **complexes** with **MD-2** in an LPS-**binding** protein- and CD14-dependent manner with similar efficiency. However, msbB LOS:**MD-2** and acyloxyacylhydrolase-treated LOS:**MD-2** were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:**MD-2** and partially antagonized the action of wt LOS:**MD-2**. These findings suggest that underacylated **endotoxins** produce decreased

TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2 complex** with TLR4 in a way that reduces receptor activation. Differences in potency among these **endotoxin** species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin: MD-2 complexes.**

CT Acylation
Antigens, CD14: PH, physiology
Cell Line
Comparative Study
***Endotoxins: AI, antagonists & inhibitors**
***Endotoxins: ME, metabolism**
Endotoxins: TO, toxicity
Humans
Lipopolysaccharides: ME, metabolism
Neisseria meningitidis: ME, metabolism
Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.

L60 ANSWER 24 OF 50 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2005211589 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15845500
TITLE: Differential induction of the toll-like receptor 4-MyD88-dependent and -independent signaling pathways by endotoxins.
AUTHOR: Zughaier Susu M; Zimmer Shanta M; Datta Anup; Carlson Russell W; Stephens David S
CORPORATE SOURCE: Division of Infectious Diseases, Emory University School of Medicine, VAMC (I-151), 1670 Clairmont Rd, Atlanta, GA 30033, USA.. szughai@emory.edu
CONTRACT NUMBER: R01 AI033517-10 (NIAID)
SOURCE: Infection and immunity, (2005 May) Vol. 73, No. 5, pp. 2940-50.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 23 Apr 2005
Last Updated on STN: 8 Jun 2005
Entered Medline: 7 Jun 2005

AB The biological response to **endotoxin** mediated through the Toll-like receptor 4 (TLR4)-**MD-2** receptor **complex** is directly related to lipid A structure or configuration. Endotoxin structure may also influence activation of the MyD88-dependent and -independent signaling pathways of TLR4. To address this possibility, human macrophage-like cell lines (THP-1, U937, and MM6) or murine macrophage RAW 264.7 cells were stimulated with picomolar concentrations of highly purified endotoxins. Harvested supernatants from previously stimulated cells were also used to stimulate RAW 264.7 or 23ScCr (TLR4-deficient) macrophages (i.e., indirect induction). Neisseria meningitidis lipooligosaccharide (LOS) was a potent direct inducer of the MyD88-dependent pathway molecules tumor necrosis factor alpha (TNF-alpha), interleukin-1beta (IL-1beta), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 3alpha (MIP-3alpha), and the MyD88-independent molecules beta interferon (IFN-beta), nitric oxide, and IFN-gamma-inducible protein 10 (IP-10). Escherichia coli 55:B5 and Vibrio cholerae lipopolysaccharides (LPSs) at the same pmole/ml lipid A

concentrations induced comparable levels of TNF-alpha, IL-1beta, and MIP-3alpha, but significantly less IFN-beta, nitric oxide, and IP-10. In contrast, LPS from Salmonella enterica serovars Minnesota and Typhimurium induced amounts of IFN-beta, nitric oxide, and IP-10 similar to meningococcal LOS but much less TNF-alpha and MIP-3alpha in time course and dose-response experiments. No MyD88-dependent or -independent response to **endotoxin** was seen in TLR4-deficient cell lines (C3H/HeJ and 23ScCr) and response was restored in TLR4-**MD**-**2**-transfected human embryonic kidney 293 cells. Blocking the MyD88-dependent pathway by DNMyD88 resulted in significant reduction of TNF-alpha release but did not influence nitric oxide release. IFN-beta polyclonal antibody and IFN-alpha/beta receptor 1 antibody significantly reduced nitric oxide release. N. meningitidis endotoxin was a potent agonist of both the MyD88-dependent and -independent signaling pathways of the TLR4 receptor **complex** of human macrophages. E. coli 55:B5 and Vibrio cholerae LPS, at the same picomolar lipid A concentrations, selectively induced the MyD88-dependent pathway, while Salmonella LPS activated the MyD88-independent pathway.

CT Adaptor Proteins, Signal Transducing
Animals
*Antigens, Differentiation: ME, metabolism
Cell Line
Cytokines: ME, metabolism
***Endotoxins: CH, chemistry**
***Endotoxins: PH, physiology**
Gram-Negative Bacteria: IM, immunology
Gram-Negative Bacteria: ME, metabolism
Gram-Negative Bacteria: PY, pathogenicity
Humans
Lipid A: CH, chemistry
Lipid A: PD, pharmacology
Lipopolysaccharides: CH, chemistry
Macrophage Activation: DE, drug effects
*Macrophage Activation: IM, immunology
Macrophages: IM, immunology
Macrophages: ME, metabolism
*Membrane Glycoproteins: ME, metabolism
Mice
Mice, Inbred C3H
Nitric Oxide: ME, metabolism
*Receptors, Cell Surface: ME, metabolism
*Receptors, Immunologic: ME, metabolism
Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, P.H.S.
*Signal Transduction
Toll-Like Receptor 4
Toll-Like Receptors

L60 ANSWER 25 OF 50 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2005303694 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15949133
TITLE: Detoxifying endotoxin: time, place and person.
AUTHOR: Munford Robert S
CORPORATE SOURCE: Molecular Host Defense Laboratory, Departments of Internal Medicine and Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75390, USA.. robert.munford@utsouthwestern.edu
CONTRACT NUMBER: AI8188 (NIAID)
SOURCE: Journal of endotoxin research, (2005) Vol. 11, No. 2, pp. 69-84. Ref: 166

Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 14 Jun 2005
Last Updated on STN: 3 Aug 2005
Entered Medline: 2 Aug 2005

AB Animals that cannot sense endotoxin may die if they are infected by Gram-negative bacteria. Animals that sense endotoxin and respond too vigorously may also die, victims of their own inflammatory reactions. The outcome of Gram-negative bacterial infection is thus determined not only by an individual's ability to sense endotoxin and respond to its presence, but also by numerous phenomena that inactivate endotoxin and/or prevent harmful reactions to it. **Endotoxin** sensing requires the **MD-2**/TLR4 recognition **complex** and occurs principally in local tissues and the liver. This review highlights the known detoxification mechanisms, which include: (i) proteins that facilitate LPS sequestration by plasma lipoproteins, prevent interactions between the bioactive lipid A moiety and **MD-2**/TLR4, or promote cellular uptake via non-signaling pathway(s); (ii) enzymes that deacylate or dephosphorylate lipid A; (iii) mechanisms that remove LPS and Gram-negative bacteria from the bloodstream; and (iv) neuroendocrine adaptations that modulate LPS-induced mediator production or neutralize pro-inflammatory molecules in the circulation. In general, the mechanisms for sensing and detoxifying endotoxin seem to be compartmentalized (local versus systemic), dynamic, and variable between individuals. They may have evolved to confine infection and inflammation to extravascular sites of infection while preventing harmful systemic reactions. Integration of endotoxin sensing and detoxification is essential for successful host defense.

CT Animals
Bacterial Infections: ME, metabolism
***Endotoxins: ME, metabolism**
Endotoxins: TO, toxicity
Humans
Lipid A: ME, metabolism
Research Support, N.I.H., Extramural
Research Support, U.S. Gov't, P.H.S.
Reticuloendothelial System: ME, metabolism

L60 ANSWER 26 OF 50 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 2004000596 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14688118
TITLE: Neisseria meningitidis lipooligosaccharide structure-dependent activation of the macrophage CD14/Toll-like receptor 4 pathway.
AUTHOR: Zughaier Susu M; Tzeng Yih-Ling; Zimmer Shanta M; Datta Anup; Carlson Russell W; Stephens David S
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA.
CONTRACT NUMBER: 2 R01 AI033517-10 (NIAID)
SOURCE: Infection and immunity, (2004 Jan) Vol. 72, No. 1, pp. 371-80.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200402
ENTRY DATE: Entered STN: 3 Jan 2004
Last Updated on STN: 3 Feb 2004
Entered Medline: 2 Feb 2004

AB Meningococcal lipopoly(oligo)saccharide (LOS) is a major inflammatory mediator of fulminant meningococcal sepsis and meningitis. Highly purified wild-type meningococcal LOS and LOS from genetically defined mutants of Neisseria meningitidis that contained specific mutations in LOS biosynthesis pathways were used to confirm that meningococcal LOS activation of macrophages was CD14/Toll-like receptor 4 (TLR4)-MD-2 dependent and to elucidate the LOS structural requirement for TLR4 activation. Expression of TLR4 but not TLR2 was required, and antibodies to both TLR4 and CD14 blocked meningococcal LOS activation of macrophages. Meningococcal LOS alpha or beta chain oligosaccharide structure did not influence CD14/TLR4-MD-2 activation. However, meningococcal lipid A, expressed by meningococci with defects in 3-deoxy-D-manno-octulosonic acid (KDO) biosynthesis or transfer, resulted in an approximately 10-fold ($P < 0.0001$) reduction in biologic activity compared to KDO2-containing meningococcal LOS. Removal of KDO2 from LOS by acid hydrolysis also dramatically attenuated cellular responses. Competitive inhibition assays showed similar **binding** of glycosylated and unglycosylated lipid A to CD14/TLR4-MD-2. A decrease in the number of lipid A phosphate head groups or penta-acylated meningococcal LOS modestly attenuated biologic activity. Meningococcal **endotoxin** is a potent agonist of the macrophage CD14/TLR4-**MD-2** receptor, helping explain the fulminant presentation of meningococcal sepsis and meningitis. KDO2 linked to meningococcal lipid A was structurally required for maximal activation of the human macrophage TLR4 pathway and indicates an important role for KDO-lipid A in endotoxin biologic activity.

CT Animals
*Antigens, CD14: ME, metabolism
Antigens, Surface: ME, metabolism
Cell Line
Humans
Lipid A: CH, chemistry
*Lipopolysaccharides: CH, chemistry
*Lipopolysaccharides: IM, immunology
Lymphocyte Antigen 96
*Macrophage Activation
Macrophages: IM, immunology
Macrophages: ME, metabolism
*Membrane Glycoproteins: ME, metabolism
Mice
*Neisseria meningitidis: IM, immunology
*Receptors, Cell Surface: ME, metabolism
Research Support, U.S. Gov't, P.H.S.
Respiratory Burst
Structure-Activity Relationship
Sugar Acids: CH, chemistry
Toll-Like Receptor 2
Toll-Like Receptor 4
Toll-Like Receptors
U937 Cells

L60 ANSWER 27 OF 50 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 2004223026 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15119998
TITLE: Molecular mechanisms of endotoxin tolerance.
AUTHOR: Fan Hongkuan; Cook James A

CORPORATE SOURCE: Department of Physiology and Neuroscience, Medical University of South Carolina, Charleston, South Carolina 29425, USA.
CONTRACT NUMBER: GM27673 (NIGMS)
SOURCE: Journal of endotoxin research, (2004) Vol. 10, No. 2, pp. 71-84. Ref: 128
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200412
ENTRY DATE: Entered STN: 5 May 2004
Last Updated on STN: 19 Dec 2004
Entered Medline: 8 Dec 2004

AB The phenomenon of endotoxin tolerance has been widely investigated, but to date, the molecular mechanisms of endotoxin tolerance remain to be resolved clearly. The discovery of the Toll-like receptor (TLR) family as the major receptors for lipopolysaccharide (LPS) and other bacterial products has prompted a resurgence of interest in endotoxin tolerance mechanisms. Changes of cell surface molecules, signaling proteins, pro-inflammatory and anti-inflammatory cytokines and other mediators have been examined. During tolerance expression of LPS-binding protein (LBP), CD14, myeloid differentiation protein-2 (**MD-2**) and TLR2 are unchanged or up-regulated, whereas TLR4 is transiently suppressed or unchanged. Proximal post-receptor signaling proteins that are altered in tolerance include augmented degradation of interleukin-1 receptor-associated kinase (IRAK), and decreased TLR4-myeloid differentiation factor 88 (MyD88) and IRAK-MyD88 association. Tolerance has also been shown to be associated with decreased Gi protein content and activity, decreased protein kinase C (PKC) activity, reduction in mitogen-activated protein kinase (MAP kinase) activity, and reduced activator protein-1 (AP-1) and nuclear factor kappa B (NF-kappaB) induced gene transactivation. However, not all signaling proteins and pathways are suppressed in tolerance and induction of specific anti-inflammatory proteins and signaling pathways may serve important counter inflammatory functions. The latter include induction of IRAK-M and suppressor of cytokine-signaling-1 (SOCS-1), phosphoinositide-3-kinase (PI3K) signaling, and increased or maintained expression of inhibitor-kappaB (IkappaB) isoforms. Also at the nuclear level, increase in the NF-kappaB subunit p50 homodimer expression and increased activation of peroxisome-proliferator-activated receptors-gamma (PPARgamma) have been linked to tolerance phenotype. Although there are species and cellular variations in manifestation of the LPS tolerant phenotype, it is clear that the tolerance phenomena have evolved as a **complex** orchestrated counter regulatory response to inflammation.

CT Animals
*Drug Tolerance
***Endotoxins: TO, toxicity**
Humans
*Lipopolysaccharides: TO, toxicity
Research Support, U.S. Gov't, P.H.S.

L60 ANSWER 28 OF 50 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 2003554314 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12960171
TITLE: Lysines 128 and 132 enable lipopolysaccharide **binding** to MD-2, leading to Toll-like receptor-4 aggregation and signal transduction.

AUTHOR: Visintin Alberto; Latz Eicke; Monks Brian G; Espevik Terje; Golenbock Douglas T
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA.
CONTRACT NUMBER: DK50305 (NIDDK)
GM54060 (NIGMS)
GM63244 (NIGMS)
SOURCE: The Journal of biological chemistry, (2003 Nov 28) Vol. 278, No. 48, pp. 48313-20. Electronic Publication: 2003-09-05.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 25 Nov 2003
Last Updated on STN: 13 Jan 2004
Entered Medline: 12 Jan 2004

AB Three cell-surface proteins have been recognized as components of the mammalian signaling receptor for bacterial lipopolysaccharide (LPS): CD14, Toll-like receptor-4 (TLR4), and MD-2. Biochemical and visual studies shown here demonstrate that the role of CD14 in signal transduction is to enhance LPS **binding** to MD-2, although its expression is not essential for cellular activation. These studies clarify how MD-2 functions: we found that MD-2 enables TLR4 **binding** to LPS and allows the formation of stable receptor **complexes.** MD-2 must be **bound** to TLR4 on the cell surface before **binding** can occur. Consequently, TLR4 clusters into receptosomes (many of which are massive) that recruit intracellular toll/IL-1/resistance domain-containing adapter proteins within minutes, thus initiating signal transduction. TLR4 activation correlates with the ability of MD-2 to **bind** LPS, as MD-2 mutants that still **bind** TLR4, but are impaired in the ability to **bind** LPS, conferred a greatly blunted LPS response. These findings help clarify the earliest events of TLR4 triggering by LPS and identify **MD-2** as an attractive target for pharmacological intervention in **endotoxin**-mediated diseases.

CT Amino Acid Sequence
Antigens, CD14: BI, biosynthesis
Antigens, CD14: ME, metabolism
*Antigens, Surface: ME, metabolism
Biotinylation
Blotting, Western
Cell Line
Cell Membrane: ME, metabolism
Cell Membrane: UL, ultrastructure
Cysteine: CH, chemistry
Humans
*Lipopolysaccharides: ME, metabolism
Lymphocyte Antigen 96
*Lysine: CH, chemistry
*Membrane Glycoproteins: ME, metabolism
Microscopy, Electron, Scanning
Microscopy, Fluorescence
Molecular Sequence Data
Precipitin Tests
Protein Binding
Protein Structure, Tertiary
*Receptors, Cell Surface: ME, metabolism

Recombinant Proteins: ME, metabolism
Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.
Sequence Homology, Amino Acid
*Signal Transduction
Toll-Like Receptor 4
Toll-Like Receptors
Transfection
Tyrosine: CH, chemistry

L60 ANSWER 29 OF 50 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 2003468338 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14517279
TITLE: Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: higher affinity than that with MD-2 or CD14.
AUTHOR: Akashi Sachiko; Saitoh Shin-ichiroh; Wakabayashi Yasutaka; Kikuchi Takane; Takamura Noriaki; Nagai Yoshinori; Kusumoto Yutaka; Fukase Koichi; Kusumoto Shoichi; Adachi Yoshiyuki; Kosugi Atsushi; Miyake Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan.
SOURCE: The Journal of experimental medicine, (2003 Oct 6) Vol. 198, No. 7, pp. 1035-42. Electronic Publication: 2003-09-29.
Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 8 Oct 2003
Last Updated on STN: 13 Nov 2003
Entered Medline: 12 Nov 2003

AB Toll-like receptors (TLRs) are innate recognition molecules for microbial products, but their direct interactions with corresponding ligands remain unclarified. LPS, a membrane constituent of gram-negative bacteria, is the best-studied TLR ligand and is recognized by TLR4 and MD-2, a molecule associated with the extracellular domain of TLR4. Although TLR4-MD-2 recognizes LPS, little is known about the physical interaction between LPS and TLR4-MD-2. Here, we demonstrate cell surface LPS-TLR4-MD-2 **complexes.** CD14 greatly enhances the formation of LPS-TLR4-MD-2 **complexes**, but is not coprecipitated with LPS-TLR4-MD-2 **complexes**, suggesting a role for CD14 in LPS loading onto TLR4-MD-2 but not in the interaction itself between LPS and TLR4-MD-2. A tentative dissociation constant (Kd) for LPS-TLR4-MD-2 **complexes** was approximately 3 nM, which is approximately 10-20 times lower than the reported Kd for LPS-MD-2 or LPS-CD14. The presence of detergent disrupts LPS interaction with CD14 but not with TLR4-MD-2. E5531, a lipid A antagonist developed for therapeutic intervention of **endotoxin** shock, blocks LPS interaction with TLR4-**MD-2** at a concentration 100 times lower than that required for blocking LPS interaction with CD14. These results reveal direct LPS interaction with cell surface TLR4-MD-2 that is distinct from that with MD-2 or CD14.

CT Animals
Antibodies, Monoclonal: IM, immunology
*Antigens, CD14: PH, physiology
*Antigens, Surface: ME, metabolism
*Lipid A: AA, analogs & derivatives

Lipid A: AI, antagonists & inhibitors
Lipid A: ME, metabolism
Lipid A: PD, pharmacology
*Lipopolysaccharides: ME, metabolism
Lymphocyte Antigen 96
*Membrane Glycoproteins: ME, metabolism
Mice
*Receptors, Cell Surface: ME, metabolism
Research Support, Non-U.S. Gov't
Toll-Like Receptor 4
Toll-Like Receptors

L60 ANSWER 30 OF 50 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 2004038098 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14733729
TITLE: Regulation of interactions of endotoxin with host cells.
AUTHOR: Gioannini Theresa L; Teghanemt Athmane; Zarember Kol A; Weiss Jerrold P
CORPORATE SOURCE: Department of Internal Medicine, Division of Infectious Diseases and The Inflammation Program, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242, USA.
CONTRACT NUMBER: DK 05472 (NIDDK)
P01 44642
SOURCE: Journal of endotoxin research, (2003) Vol. 9, No. 6, pp. 401-8.
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 24 Jan 2004
Last Updated on STN: 17 Aug 2004
Entered Medline: 16 Aug 2004

AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by **endotoxin** requires lipopolysaccharide-**binding** protein (LBP) and CD14-dependent delivery of **endotoxin** to cells containing **MD-2** and TLR4. We have used metabolically labeled [(14)C] meningococcal lipooligosaccharide (LOS), purified recombinant endotoxin-**binding** proteins, and cultured endothelial cells to better define protein:endotoxin intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:endotoxin **complexes** or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of endotoxin agg (M(r) > or = 20 x 10(6)) to monomeric (M(r) approximately 55 x 10(3)) endotoxin:sCD14 **complexes.** Ordered interaction of LBP (+ albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive endotoxin:sCD14 **complex** and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of endotoxin:sCD14 **complexes** and instead yielded aggregates of endotoxin (M(r) approximately 1-20 x 10(6)) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of endotoxin selectively recognizes different protein:endotoxin **complexes.** At the outset of infection, the low concentrations of

LBP present and absence of extracellular BPI favor formation of pro-inflammatory endotoxin:CD14 **complexes.** The mobilization of LBP and BPI that is triggered by inflammation directs endotoxin for clearance and hence resolution of endotoxin-triggered inflammation.

CT Albumins: PH, physiology
Antibodies, Monoclonal: ME, metabolism
Antigens, CD14: IM, immunology
Antigens, CD14: ME, metabolism
Carbon Radioisotopes
Cell Line
Chromatography, Gel
Dose-Response Relationship, Drug
*Endothelial Cells: ME, metabolism
***Endotoxins: ME, metabolism**
Humans
Membrane Glycoproteins: IM, immunology
Membrane Glycoproteins: ME, metabolism
Models, Biological
Mutagenesis, Site-Directed
Neisseria meningitidis: ME, metabolism
Protein Kinases: GE, genetics
Receptors, Cell Surface: IM, immunology
Receptors, Cell Surface: ME, metabolism
Recombinant Proteins: ME, metabolism
Research Support, U.S. Gov't, P.H.S.
Toll-Like Receptor 4
Toll-Like Receptors
Umbilical Veins: CY, cytology

L60 ANSWER 31 OF 50 MEDLINE on STN DUPLICATE 18
ACCESSION NUMBER: 2003173923 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12691621
TITLE: Overexpression of CD14, TLR4, and **MD-2** in HEK 293T cells does not prevent induction of in vitro **endotoxin** tolerance.
AUTHOR: Medvedev Andrei E; Vogel Stefanie N
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, Baltimore 21201, USA.
CONTRACT NUMBER: AI-18797 (NIAID)
AI-44936 (NIAID)
SOURCE: Journal of endotoxin research, (2003) Vol. 9, No. 1, pp. 60-4.
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 16 Apr 2003
Last Updated on STN: 22 Aug 2003
Entered Medline: 21 Aug 2003

AB TLR4 and MD-2 are necessary for conferring cellular responsiveness to LPS. Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS re-stimulation, known as 'endotoxin tolerance'. While induction of LPS tolerance has been reported to correlate with down-regulation of cell surface expression of TLR4/MD-2, other mechanisms of LPS tolerance have been revealed that target intracellular intermediates downstream of the TLR4/MD-2 **complex.** In this study, we sought to examine whether **endotoxin** tolerance could be induced under conditions where expression of TLR4 and **MD-**

2 proteins is not affected by LPS. Human HEK 293T cells are completely unresponsive to LPS, but acquire high LPS sensitivity following transient transfection with CD14, TLR4, and MD-2 (293T/CD14/TLR4/MD-2 cells), as judged by NF-kappaB activation, ERK 1/2 phosphorylation, and TNF-alpha gene expression. Prior exposure of 293T/CD14/TLR4/MD-2 cells to LPS resulted in a significant decrease of LPS-mediated responses, yet failed to affect expression levels of TLR4 and MD-2. Thus, altered expression and/or function of intracellular mediators downstream of the TLR4/MD-2 **complex** play an important role in mediating LPS tolerance.

CT *Antigens, CD14: ME, metabolism
*Antigens, Surface: ME, metabolism
Blotting, Western
Cell Line: DE, drug effects
Cell Line: IM, immunology
Cell Line: ME, metabolism
Dose-Response Relationship, Drug
Down-Regulation: IM, immunology
Drug Tolerance
Escherichia coli: IM, immunology
Gene Expression
Humans
*Immune Tolerance
Immune Tolerance: DE, drug effects
Immune Tolerance: IM, immunology
Kidney
Lipopolysaccharides: PD, pharmacology
Lymphocyte Antigen 96
*Membrane Glycoproteins: ME, metabolism
Mitogen-Activated Protein Kinase 1: GE, genetics
Mitogen-Activated Protein Kinase 1: ME, metabolism
Mitogen-Activated Protein Kinase 3
Mitogen-Activated Protein Kinases: GE, genetics
Mitogen-Activated Protein Kinases: ME, metabolism
NF-kappa B: GE, genetics
NF-kappa B: ME, metabolism
Phosphorylation
RNA, Messenger: ME, metabolism
*Receptors, Cell Surface: ME, metabolism
Research Support, U.S. Gov't, P.H.S.
Reverse Transcriptase Polymerase Chain Reaction
Toll-Like Receptor 4
Toll-Like Receptors
Transfection
Tumor Necrosis Factor-alpha: GE, genetics
Tumor Necrosis Factor-alpha: ME, metabolism

L60 ANSWER 32 OF 50 MEDLINE on STN DUPLICATE 19
ACCESSION NUMBER: 2002641132 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12391239
TITLE: Dysregulation of LPS-induced Toll-like receptor 4-MyD88 **complex** formation and IL-1 receptor-associated kinase 1 activation in endotoxin-tolerant cells.
AUTHOR: Medvedev Andrei E; Lentschat Arnd; Wahl Larry M; Golenbock Douglas T; Vogel Stefanie N
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Maryland, Baltimore 21201, USA.
CONTRACT NUMBER: AI18797 (NIAID)
AI44936 (NIAID)
AIP0150305 (NIAID)

R01GM54060 (NIGMS)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2002 Nov 1) Vol. 169, No. 9, pp. 5209-16.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 29 Oct 2002
Last Updated on STN: 17 Dec 2002
Entered Medline: 10 Dec 2002

AB Prior exposure to LPS induces a transient state of cell refractoriness to subsequent LPS restimulation, known as endotoxin tolerance. Induction of LPS tolerance has been reported to correlate with decreased cell surface expression of the LPS receptor **complex**, Toll-like receptor 4 (TLR4)/MD-2. However, other results have underscored the existence of mechanisms of LPS tolerance that operate downstream of TLR4/MD-2. In the present study we sought to delineate further the molecular basis of LPS tolerance by examining the TLR4 signaling pathway in endotoxin-tolerant cells. Pretreatment of human monocytes with LPS decreased LPS-mediated NF-kappaB activation, p38 mitogen-activated protein kinase phosphorylation, and TNF-alpha gene expression, documenting the induction of endotoxin tolerance. FACS and Western blot analyses of LPS-tolerant monocytes showed increased TLR2 expression, whereas TLR4 expression levels were not affected. Comparable levels of mRNA and protein for myeloid differentiation factor 88 (MyD88), IL-1R-associated kinase 1 (IRAK-1), and TNFR-associated factor-6 were found in normal and LPS-tolerant monocytes, while MD-2 mRNA expression was slightly increased in LPS-tolerant cells. LPS induced the association of MyD88 with TLR4 and increased IRAK-1 activity in medium-pretreated cells. In LPS-tolerant monocytes, however, MyD88 failed to be recruited to TLR4, and IRAK-1 was not activated in response to LPS stimulation. Moreover, **endotoxin**-tolerant CHO cells that overexpress human TLR4 and **MD-2** also showed decreased IRAK-1 kinase activity in response to LPS despite the failure of LPS to inhibit cell surface expression of transfected TLR4 and **MD-2** proteins. Thus, decreased TLR4-MyD88 **complex** formation with subsequent impairment of IRAK-1 activity may underlie the LPS-tolerant phenotype.

CT Adaptor Proteins, Signal Transducing
Animals
Antigens, Differentiation: ME, metabolism
CHO Cells
Cricetinae
*Down-Regulation: IM, immunology
*Drosophila Proteins
Enzyme Activation: IM, immunology
Enzyme Inhibitors: PD, pharmacology
Humans
*Immune Tolerance
Intracellular Fluid: IM, immunology
Intracellular Fluid: ME, metabolism
*Lipopolysaccharides: PD, pharmacology
Macromolecular Substances
*Membrane Glycoproteins: AI, antagonists & inhibitors
Membrane Glycoproteins: BI, biosynthesis
Membrane Glycoproteins: ME, metabolism
Membrane Glycoproteins: PH, physiology
Monocytes: EN, enzymology
Monocytes: IM, immunology

Monocytes: ME, metabolism
Phosphorylation
*Protein Kinase Inhibitors
Protein Kinases: ME, metabolism
RNA, Messenger: BI, biosynthesis
*Receptors, Cell Surface: AI, antagonists & inhibitors
Receptors, Cell Surface: BI, biosynthesis
Receptors, Cell Surface: ME, metabolism
Receptors, Cell Surface: PH, physiology
*Receptors, Immunologic: AI, antagonists & inhibitors
Receptors, Immunologic: ME, metabolism
*Receptors, Interleukin-1: ME, metabolism
Research Support, U.S. Gov't, P.H.S.
Signal Transduction: IM, immunology
Toll-Like Receptor 2
Toll-Like Receptor 4
Toll-Like Receptors

L60 ANSWER 33 OF 50 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 2002387284 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12135807
TITLE: Initial responses to endotoxins and Gram-negative bacteria.
AUTHOR: Heumann Didier; Roger Thierry
CORPORATE SOURCE: Department of Internal Medicine, Division of Infectious Diseases, BH19-111, Centre Hospitalier Universitaire Vaudois, rue du Bugnon 46, CH-1011, Lausanne, Switzerland.. didier.heumann@bluewin.ch
SOURCE: Clinica chimica acta; international journal of clinical chemistry, (2002 Sep) Vol. 323, No. 1-2, pp. 59-72. Ref: 92
Journal code: 1302422. ISSN: 0009-8981.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 24 Jul 2002
Last Updated on STN: 10 Oct 2002
Entered Medline: 8 Oct 2002

AB The innate immune system initiates host defence against invasive microbial pathogens using specific recognition mechanisms. Here we review the current concepts and the molecular basis of innate immune responses to bacterial infections, focusing our attention on the actors involved in the response to Gram-negative bacteria.Lipopolysaccharide (LPS) is the major virulence factor of Gram-negative bacteria. During the past decade, enormous progress has been obtained in the elucidation of LPS recognition and signalling in mammalian phagocytes. According to the current model, recognition of LPS is initialized by the cooperative interplay between the LPS-binding protein (LBP), the membrane-bound or soluble forms of CD14 and the recently identified Toll-like receptor 4 (TLR4)-**MD-2 complex.** Recognition of LPS leads to the rapid activation of an intracellular signalling pathway, highly homologous to the signalling pathway of interleukin-1, which results in the release of pro-inflammatory mediators.In vivo models in which animals are challenged with LPS or Gram-negative bacteria have highlighted opposite roles for LBP, CD14 and TLRs. Regarding LPS challenge, there is a large body of evidence in favour of a detrimental role played by LBP, CD14 and TLRs. These molecules sensitize the host to a LPS-induced uncontrolled acute inflammatory response that results in animal death. However, when the

host is in the presence of virulent Gram-negative bacteria, the invading pathogens must be held in check by the innate immune system until a specific immune response is mounted. Under these conditions, LBP, CD14 and TLRs are required to trigger a pro-inflammatory response which is crucial for keeping infection under control. Therefore, caution should be the rule about the development of therapeutic approaches aimed at blocking the pro-inflammatory response during Gram-negative infections.

CT *Acute-Phase Proteins
Animals
Antigens, CD14: ME, metabolism
Carrier Proteins: ME, metabolism
*Drosophila Proteins
***Endotoxins: IM, immunology**
*Gram-Negative Bacteria: IM, immunology
*Gram-Negative Bacterial Infections: IM, immunology
Humans
Immunity, Natural
Lipopolysaccharides: IM, immunology
Macrophages: IM, immunology
Membrane Glycoproteins: ME, metabolism
Receptors, Cell Surface: ME, metabolism
Research Support, Non-U.S. Gov't
Toll-Like Receptor 4
Toll-Like Receptors

L60 ANSWER 34 OF 50 MEDLINE on STN
ACCESSION NUMBER: 2004342289 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15121639
TITLE: **Endotoxin** responsiveness of human airway epithelia is limited by low expression of **MD-2.**
AUTHOR: Jia Hong Peng; Kline Joel N; Penisten Andrea; Apicella Michael A; Gioannini Theresa L; Weiss Jerrold; McCray Paul B Jr
CORPORATE SOURCE: Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA.
CONTRACT NUMBER: AI-24616 (NIAID)
AI-44642 (NIAID)
AI-65298 (NIAID)
ES-005605 (NIEHS)
HL-59324 (NHLBI)
HL-62134 (NHLBI)
P30 DK-54759 (NIDDK)
SOURCE: American journal of physiology. Lung cellular and molecular physiology, (2004 Aug) Vol. 287, No. 2, pp. L428-37. Electronic Publication: 2004-04-30. Journal code: 100901229. ISSN: 1040-0605.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 13 Jul 2004
Last Updated on STN: 18 Aug 2004
Entered Medline: 17 Aug 2004

AB The expression of inducible antimicrobial peptides, such as human beta-defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the Toll-like receptors (TLRs). We found that primary cultures of well-differentiated

human airway epithelia express the mRNA for TLR-4, but little or no **MD-2** mRNA, and display little HBD-2 expression in response to treatment with purified **endotoxin** +/- LPS **binding** protein (LBP) and soluble CD14. Expression of endogenous **MD-2** by transduction of airway epithelial cells with an adenoviral vector encoding **MD-2** or extracellular addition of recombinant **MD-2** both increased the responses of airway epithelia to **endotoxin** + LBP and sCD14 by >100-fold, as measured by NF-kappaB-luciferase activity and HBD-2 mRNA expression. MD-2 mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed Haemophilus influenzae, the P6 outer membrane protein from H. influenzae, or TNF-alpha + IFN-gamma). These findings suggest that **MD-2**, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to **endotoxin.** The regulation of **MD-2** expression in airway epithelia and pulmonary macrophages may serve as a means to modify **endotoxin** responsiveness in the airway.

CT *Antigens, Surface: GE, genetics
Cells, Cultured
*Endotoxins: PD, pharmacology
Extracellular Space: IM, immunology
Gene Expression: DE, drug effects
Humans
Kidney: CY, cytology
Lymphocyte Antigen 96
Macrophages, Alveolar: IM, immunology
Pneumonia: IM, immunology
Pneumonia: PP, physiopathology
Research Support, Non-U.S. Gov't
Research Support, U.S. Gov't, P.H.S.
Respiratory Mucosa: CY, cytology
*Respiratory Mucosa: DE, drug effects
*Respiratory Mucosa: PH, physiology
Signal Transduction: IM, immunology

L60 ANSWER 35 OF 50 MEDLINE on STN
ACCESSION NUMBER: 2004375473 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15276183
TITLE: **MD-2**: the Toll 'gatekeeper' in **endotoxin** signalling.
AUTHOR: Gangloff Monique; Gay Nicholas J
CORPORATE SOURCE: Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK.. mg308@cam.ac.uk
SOURCE: Trends in biochemical sciences, (2004 Jun) Vol. 29, No. 6, pp. 294-300.
Journal code: 7610674. ISSN: 0968-0004.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200411
ENTRY DATE: Entered STN: 28 Jul 2004
Last Updated on STN: 10 Nov 2004
Entered Medline: 9 Nov 2004

AB Lipopolysaccharide (LPS) from the outer cell wall of Gram-negative bacteria is a potent stimulator of the mammalian innate immune system. The Toll-like receptor 4 (TLR4) pathway triggers the inflammatory responses induced by LPS in a process that requires the interaction of

LPS-**bound** myeloid differentiation-2 (**MD-2**) with TLR4. Here we propose two possible mechanisms for LPS recognition and signalling that take into account both the structural information available for TLR4 and **MD-2**, and the determinants of endotoxicity, namely, the acylation and phosphorylation patterns of LPS. In our first model, LPS induces the association of two TLR4-**MD-2** heterodimers by **binding** to two different molecules of **MD-2** through the acyl chains of lipid A. In our second model, the **binding** of LPS to a single TLR4-**MD-2 complex** facilitates the recruitment of a second TLR4-**MD-2** heterodimer. These models contrast with the activation of Drosophila Toll, where the receptor is crosslinked by a dimeric protein ligand.

CT Animals
*Antigens, Surface: ME, metabolism
Carbohydrate Sequence
Drosophila
Drosophila Proteins: ME, metabolism
***Endotoxins: ME, metabolism**
Humans
Lipopolysaccharides: ME, metabolism
Lymphocyte Antigen 96
Membrane Glycoproteins: ME, metabolism
Models, Biological
Molecular Sequence Data
Protein Structure, Tertiary
Receptors, Cell Surface: ME, metabolism
Research Support, Non-U.S. Gov't
Signal Transduction
Substrate Specificity
Toll-Like Receptor 4
Toll-Like Receptors

L60 ANSWER 36 OF 50 MEDLINE on STN
ACCESSION NUMBER: 2003542158 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14615419
TITLE: Lipopolysaccharide activates nuclear factor-kappaB through toll-like receptors and related molecules in cultured biliary epithelial cells.
AUTHOR: Harada Kenichi; Ohira Shusaku; Isse Kumiko; Ozaki Satoru; Zen Yoh; Sato Yasunori; Nakanuma Yasuni
CORPORATE SOURCE: Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan.
SOURCE: Laboratory investigation; a journal of technical methods and pathology, (2003 Nov) Vol. 83, No. 11, pp. 1657-67. Journal code: 0376617. ISSN: 0023-6837.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 19 Nov 2003
Last Updated on STN: 19 Dec 2003
Entered Medline: 2 Dec 2003

AB To clarify the innate immunity of the intrahepatic biliary tree, we examined expression of Toll-like receptors and intracellular signalings in biliary epithelial cells in response to bacterial components by using cultured biliary epithelial cells (murine biliary cells and human cholangiocarcinoma cell lines). The expression of Toll-like receptors in cultured cells was examined by reverse transcription and PCR and

immunohistochemistry. Intracellular signalings after Toll-like receptors activation by lipopolysaccharide was examined by analysis of nuclear factor (NF)-kappaB activation and inhibition studies using inhibitors for NF-kappaB and mitogen-activated protein kinase and blocking antibody. The mRNAs of Toll-like receptors 2, 3, 4, and 5, and related molecules (**MD-2**, MyD88, and CD14) were detected, and their proteins were expressed in cultured cells. Lipopolysaccharide was shown to bind to the cell surface of cultured cells. Lipopolysaccharide treatment induced the production of TNF-alpha, and nuclear translocation of NF-kappaB and increased NF-kappaB-DNA binding in cultured cells. This induction of TNF-alpha was partially inhibited by anti-Toll-like receptor 4 antibody. The nuclear translocation and increased binding of NF-kappaB by lipopolysaccharide were blocked by addition of MG132, an inhibitor of NF-kappaB. In conclusion, lipopolysaccharide appears to form a receptor **complex** of CD14, Toll-like receptor 4, **MD-2**, and MyD88 in cultured biliary epithelial cells and seems to regulate activation of NF-kappaB and synthesis of TNF-alpha. The recognition of pathogen-associated molecular patterns using Toll-like receptors and related molecules in biliary epithelial cells, which is demonstrated in this in vitro study, may participate in an immunopathology of the intrahepatic biliary tree in vivo.

CT Check Tags: Female; Male
Adult
Aged
Animals
Bile: CH, chemistry
Bile Duct Neoplasms: ME, metabolism
Bile Duct Neoplasms: PA, pathology
*Bile Ducts, Intrahepatic: DE, drug effects
Bile Ducts, Intrahepatic: ME, metabolism
Bile Ducts, Intrahepatic: PA, pathology
Cell Line, Tumor
Cell Nucleus: DE, drug effects
Cell Nucleus: ME, metabolism
Cholangiocarcinoma: ME, metabolism
Cholangiocarcinoma: PA, pathology
DNA: CH, chemistry
DNA: ME, metabolism
DNA Primers: CH, chemistry
Endotoxins: AN, analysis
***Endotoxins: PD, pharmacology**
*Epithelium: DE, drug effects
Epithelium: ME, metabolism
*Escherichia coli
Humans
Leupeptins: PD, pharmacology
*Lipopolysaccharides: PD, pharmacology
Membrane Glycoproteins: GE, genetics
*Membrane Glycoproteins: ME, metabolism
Mice
Mice, Inbred BALB C
Middle Aged
NF-kappa B: AI, antagonists & inhibitors
*NF-kappa B: BI, biosynthesis
RNA, Messenger: ME, metabolism
Receptors, Cell Surface: GE, genetics
*Receptors, Cell Surface: ME, metabolism
Toll-Like Receptor 4
Toll-Like Receptors
Tumor Necrosis Factor-alpha: ME, metabolism

L60 ANSWER 37 OF 50 MEDLINE on STN
ACCESSION NUMBER: 2003354141 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12869026
TITLE: Evidence of expression of **endotoxin** receptors CD14, toll-like receptors TLR4 and TLR2 and associated molecule **MD-2** and of sensitivity to **endotoxin** (LPS) in islet beta cells.
AUTHOR: Vives-Pi M; Somoza N; Fernandez-Alvarez J; Vargas F; Caro P; Alba A; Gomis R; Labeta M O; Pujol-Borrell R
CORPORATE SOURCE: Laboratory of Immunobiology for Research and Diagnostic Applications, Transfusion Center and Tissue Bank Germans Trias i Pujol University Hospital, Badalona, Spain.. vivespi@ns.hugtip.scs.es
SOURCE: Clinical and experimental immunology, (2003 Aug) Vol. 133, No. 2, pp. 208-18.
Journal code: 0057202. ISSN: 0009-9104.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 31 Jul 2003
Last Updated on STN: 17 Sep 2003
Entered Medline: 16 Sep 2003

AB CD14, a GPI-linked membrane protein, is a component of the lipopolysaccharide (LPS) receptor **complex**, one of the pattern-recognizing receptors (PRR) expressed by myeloid lineage cells. Here we report that CD14, the functionally linked toll-like receptor molecules, TLR2 and TLR4, and the associated molecule MD-2 are expressed in endocrine cells of the human pancreatic islets. CD14 expression in human pancreatic islets was determined by immunofluorescence staining of tissue sections and primary cultures, and confirmed by flow cytometry of dispersed normal islets and SV40-transformed islet cells (HP62). The latter cells synthesized and secreted CD14 in response to lipopolysaccharide (LPS) in a time- and dose-dependent manner. Reverse transcription polymerase chain reaction (RT-PCR)-Southern was positive for CD14, TLR2, TLR4 and MD-2 in human pancreas, purified islets and HP62 cells. In vitro experiments using rat islets (also positive for CD14 by RT-PCR) and HP62 cells showed that LPS regulates glucose-dependent insulin secretion and induces inflammatory cytokines [interleukin (IL)-1alpha, IL-6 and tumour necrosis factor (TNF)-alpha]. The functional expression of CD14 and associated molecules in islet beta cells adds a new pathway that islet cells may follow to adjust their function to endotoxaemia situations and become vulnerable to the inflammatory events that occur during diabetogenic insulitis.

CT Check Tags: Female; Male
Adolescent
Adult
Antigens, CD14: GE, genetics
*Antigens, CD14: ME, metabolism
Antigens, Surface: ME, metabolism
Cells, Cultured
Dose-Response Relationship, Drug
Glucose: AI, antagonists & inhibitors
Glucose: PD, pharmacology
Humans
Insulin: SE, secretion
Islets of Langerhans: DE, drug effects
*Islets of Langerhans: ME, metabolism

Lipopolysaccharides: PD, pharmacology
Lymphocyte Antigen 96
*Membrane Glycoproteins: ME, metabolism
Middle Aged
*Receptors, Cell Surface: ME, metabolism
Research Support, Non-U.S. Gov't
Reverse Transcriptase Polymerase Chain Reaction
Species Specificity
Toll-Like Receptor 2
Toll-Like Receptor 4
Toll-Like Receptors
Tumor Cells, Cultured

L60 ANSWER 38 OF 50 MEDLINE on STN
ACCESSION NUMBER: 2001534223 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11581570
TITLE: Bacterial lipopolysaccharides and innate immunity.
AUTHOR: Alexander C; Rietschel E T
CORPORATE SOURCE: Department of Immunochemistry and Biochemical Microbiology, Centre of Medicine and Bio-Sciences, Borstel, Germany.
SOURCE: Journal of endotoxin research, (2001) Vol. 7, No. 3, pp. 167-202. Ref: 478
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 3 Oct 2001
Last Updated on STN: 31 Jan 2002
Entered Medline: 30 Jan 2002

AB Bacterial lipopolysaccharides (LPS) are the major outer surface membrane components present in almost all Gram-negative bacteria and act as extremely strong stimulators of innate or natural immunity in diverse eukaryotic species ranging from insects to humans. LPS consist of a poly- or oligosaccharide region that is anchored in the outer bacterial membrane by a specific carbohydrate lipid moiety termed lipid A. The lipid A component is the primary immunostimulatory centre of LPS. With respect to immunoactivation in mammalian systems, the classical group of strongly agonistic (highly endotoxic) forms of LPS has been shown to be comprised of a rather similar set of lipid A types. In addition, several natural or derivatised lipid A structures have been identified that display comparatively low or even no immunostimulation for a given mammalian species. Some members of the latter more heterogeneous group are capable of antagonizing the effects of strongly stimulatory LPS/lipid A forms. Agonistic forms of LPS or lipid A trigger numerous physiological immunostimulatory effects in mammalian organisms, but--in higher doses--can also lead to pathological reactions such as the induction of septic shock. Cells of the myeloid lineage have been shown to be the primary cellular sensors for LPS in the mammalian immune system. During the past decade, enormous progress has been obtained in the elucidation of the central LPS/lipid A recognition and signaling system in mammalian phagocytes. According to the current model, the specific cellular recognition of agonistic LPS/lipid A is initialized by the combined extracellular actions of LPS binding protein (LBP), the membrane-bound or soluble forms of CD14 and the newly identified Toll-like receptor 4 (TLR4)*MD-2 complex, leading to the rapid activation of an intracellular signaling network that is highly homologous to the signaling systems of IL-1 and IL-18. The elucidation of

structure-activity correlations in LPS and lipid A has not only contributed to a molecular understanding of both immunostimulatory and toxic septic processes, but has also re-animated the development of new pharmacological and immunostimulatory strategies for the prevention and therapy of infectious and malignant diseases.

CT Animals
Carbohydrate Conformation
Carbohydrate Sequence
Endotoxins
Humans
Immunity, Natural: IM, immunology
Lipopolysaccharides: CH, chemistry
*Lipopolysaccharides: IM, immunology
Mammals
Molecular Sequence Data
Phagocytes: IM, immunology
Research Support, Non-U.S. Gov't
Signal Transduction: IM, immunology

L60 ANSWER 39 OF 50 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2006-253953 [26] WPIDS
DOC. NO. CPI: C2006-082768
TITLE: New soluble Toll-like receptor 4 protein, useful as a therapeutic agent for treating endotoxin-induced inflammation.
DERWENT CLASS: B04 D16
INVENTOR(S): HYAKUSHIMA, N; KUROKI, Y; MITSUZAWA, H
PATENT ASSIGNEE(S): (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
COUNTRY COUNT: 112
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2006033481	A1	20060330	(200626)*	JA	78

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT KE LS LT LU LV MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2006033481	A1	WO 2005-JP18207	20050922

PRIORITY APPLN. INFO: JP 2004-277421 20040924
AB WO2006033481 A UPAB: 20060421
NOVELTY - A soluble Toll-like receptor 4 protein (I) (TLR4), comprising an amino acid sequence having a fully defined 608 amino acid (SEQ ID No: 1) sequence, given in the specification, or an amino acid sequence that is substantially the same as SEQ ID Number 1, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) DNA (II) encoding (I);
(2) recombinant vector (III) comprising (II);

(3) non-human host cell (IV) transformed with (III);
(4) preparing (I); and
(5) therapeutic agent (A1) comprising (I).

ACTIVITY - Antiinflammatory. In vivo analysis of soluble Toll-like receptor 4 protein (sTLR4) and **MD-2** in suppressing inflammation induced by **endotoxin** was carried out as follows. A female BALB/C mouse was anesthetized by ketamine HCl and xylazine hydrochloride. The mice injected with sTLR4 and **MD-2** protein were taken as a test group, and mice injected with sTLR2 was taken as control group. Lipopolysaccharide (LPS) (1 mu g) was dripped in trachea. After 16 hours, 1 ml of Hank's solution was used to wash bronchus alveolus. The concentration of tumor necrosis factor alpha in bronchus alveolus was measured. Results showed that inflammation induced by **endotoxin** was significantly reduced in test group.

MECHANISM OF ACTION - None given.

USE - (I) Or (A1) is useful for treating **endotoxin** induced inflammation (claimed), inflammation induced by nuclear factor kappa B activation, and interleukin-8 secretion.

DESCRIPTION OF DRAWING(S) - The figure is a graph representing the inflammatory cytokine of lung decreased by administration of soluble Toll-like receptor 4 protein and **MD-2**.

Dwg.9/9

L60 ANSWER 40 OF 50 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-372350 [38] WPIDS
DOC. NO. NON-CPI: N2005-301093
DOC. NO. CPI: C2005-115401
TITLE: New anti-TLR4-**MD-2** monoclonal antibody not exerting effect of B-cell proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide stimulation, for treating **endotoxin** shock.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): MIYAKE, K; TAKAMURA, S
PATENT ASSIGNEE(S): (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2005047330	A1	20050526	(200538)*	JA	30

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2005047330	A1	WO 2004-JP14194	20040928

PRIORITY APPLN. INFO: JP 2003-387173 20031117
AB WO2005047330 A UPAB: 20050616
NOVELTY - A monoclonal antibody (I) capable of specifically recognizing a TLR4-**MD-2** composite and not exerting effect of B-cell

proliferation inhibition and TNF production inhibition in macrophages, through in vitro lipopolysaccharide (LPS) stimulation, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an anti-mouse TLR4-**MD-2** monoclonal antibody Sa 15-21 capable recognizing an antigenic determinant of mouse TLR4 in a mouse TLR4-**MD-2** composite, in the N-terminal end;

(2) an anti-human TLR4 monoclonal antibody TF904 capable of specifically recognizing the antigenic determinant of human TLR4, in the N-terminal end;

(3) a hybridoma capable of producing an anti-human TLR4 monoclonal antibody TF904 which specifically recognizes an antigenic determinant of human TLR4 (FER ABP-10118), in the N-terminal end;

(4) a therapeutic agent (A1) of **endotoxin** shock comprising (I); and

(5) screening an agent capable of promoting **endotoxin** shock inhibitory effect or substance inhibiting **endotoxin** shock, comprising administering anti-TLR4-**MD-2** mouse monoclonal antibody Sa 15-21 capable of recognizing TLR4-**MD-2** composite and a test substance, and evaluating the grade of **endotoxin** shock in a mouse, before and after the **endotoxin** shock.

ACTIVITY - Antibacterial; Immunosuppressive. No supporting data is given.

MECHANISM OF ACTION - TRL4-**MD-2** composite antagonist.

USE - (I) Is useful for treating or preventing **endotoxin** shock (claimed).

ADVANTAGE - (I) Effectively treats **endotoxin** shock.

Dwg.0/8

L60 ANSWER 41 OF 50 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-143114 [14] WPIDS
DOC. NO. NON-CPI: N2004-114058
DOC. NO. CPI: C2004-057716
TITLE: Extracorporeal adsorption agent for removing harmful substances that induce sepsis, by treating blood obtained from mammal by passing blood through adsorption column assembly at flow rate that fluidized bed of particles is formed.
DERWENT CLASS: B04 S03
INVENTOR(S): HEEGAARD, P M H; LIHME, A O F
PATENT ASSIGNEE(S): (UPFR-N) UPFRONT CHROMATOGRAPHY AS
COUNTRY COUNT: 105
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004008138	A2	20040122	(200414)*	EN	56
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
AU 2003242509	A1	20040202	(200450)		
EP 1521624	A2	20050413	(200525)	EN	
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR					

JP 2005532130 W 20051027 (200571) 48
US 2005249724 A1 20051110 (200574)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2004008138	A2	WO 2003-DK483	20030709
AU 2003242509	A1	AU 2003-242509	20030709
EP 1521624	A2	EP 2003-763618	20030709
		WO 2003-DK483	20030709
JP 2005532130	W	WO 2003-DK483	20030709
		JP 2004-520339	20030709
US 2005249724	A1	WO 2003-DK483	20030709
		US 2005-520685	20050527

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003242509	A1 Based on	WO 2004008138
EP 1521624	A2 Based on	WO 2004008138
JP 2005532130	W Based on	WO 2004008138

PRIORITY APPLN. INFO: DK 2002-1091 20020711

AB WO2004008138 A UPAB: 20040226

NOVELTY - Extracorporeal adsorption agent (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.

DETAILED DESCRIPTION - Extracorporeal adsorption (M1), for removing harmful substances responsible of inducing sepsis caused by gram-negative or gram positive bacteria in a mammal, the extracorporeal adsorption being effected by an adsorption column assembly, where the adsorption column assembly comprising a column and an adsorption medium in the form of particles, the sedimented volume of the particles being at the most 80% of the volume of the column, the particles being characterized by carrying an affinity specific molecule with a specific affinity for the LPS portion of the gram-negative bacteria, gram-positive bacteria or harmful substances derived from the gram-positive bacteria, involves treating blood obtained from the mammal by passing the blood through the adsorption column assembly at such a flow rate that a fluidized bed of the particles is formed.

ACTIVITY - Antibacterial; Immunosuppressive.

MECHANISM OF ACTION - Removing harmful substances responsible of inducing sepsis.

The use of extracorporeal adsorption for the treatment of **endotoxin**-challenged cows was as follows. The cows weighing 500-800 kg were challenged by intravenous injection of 1000 ng lipopolysaccharide (LPS)/kg body weight. After the injection of LPS, the cow was connected to a venous-venous extracorporeal adsorption circuit, comprising a stabilized fluidized bed of polymyxin B-coated particles connected through a switch, the switch being activated by a continuous monitoring device, detecting changes in the serum concentration of haptoglobin in the blood. Clinical parameters, including rectal temperature, heat rate, respiratory frequency, and acute phase protein responses was measured up to one week after the challenge and compared between cows treated by the described extracorporeal method and in treated cows. Results showed that LPS-challenged cows treated by stand-by

extracorporeal adsorption of the animal's blood in a continuous process through a stabilized fluidized bed of polymyxin B-coated particles present with significantly less, significantly less severe and significantly more short-lived clinical signs than comparable, non-treated cows.

USE - (M1) is useful for treating (M2) sepsis caused by gram-negative or gram-positive bacteria in mammal e.g., human being, which involves obtaining blood from the mammal, treating the obtained blood by passing the blood through the adsorption column assembly at such as flow rate that a fluidized bed of the particles is formed, and reinfusing the treated blood into the same mammal. The flow rate of the blood through the column assembly is such that expansion ratio of the fluidized bed is at least 1.3, such as at least 1.5. (M2) further involves injecting the substance into the blood stream of the mammal (claimed).

DESCRIPTION OF DRAWING(S) - The figure shows the principle of continuous extracorporeal adsorption.

Dwg.3/7

L60 ANSWER 42 OF 50 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-027278 [03] WPIDS
DOC. NO. NON-CPI: N2004-021623
DOC. NO. CPI: C2004-009400
TITLE: Transgenic non human animal with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide, comprises MD-2 gene deficient chromosome which encodes toll-like receptor.
DERWENT CLASS: B04 D16 P14 S03
PATENT ASSIGNEE(S): (KAGA-N) KAGAKU GIJUTSU SHINKO JIGYODAN
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 2003319734	A	20031111	(200403)*		13

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 2003319734	A	JP 2002-130964	20020502

PRIORITY APPLN. INFO: JP 2002-130964 20020502
AB JP2003319734 A UPAB: 20040112

NOVELTY - Transgenic non-human animal (I) with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide (LPS), comprises **MD-2** gene deficient chromosome which encodes toll-like receptor 4 (TLR4).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) screening (M1) a of Gram-negative-bacterial membrane component responsive substance, involves introducing a test substance into (I), or introducing a test substance into (I) having **MD-2** gene of different animal; and

(2) diagnosing (M2) the response of different **MD-2** gene in non-human animal, involves transducing **MD-2** gene into (I) and inducing an **endotoxin** shock into (I).

USE - (I) is useful for screening of Gram-negative-bacterial membrane component responsive substance, or for diagnosing the response of different **MD-2** genes in non-human animal (claimed).

(I) is useful for developing a medical agent which is used for further

drug development.

ADVANTAGE - (I) enables to screen Gram-negative-bacterial membrane component responsive substance, or to diagnose the response of different **MD-2** gene in non-human animal.

DESCRIPTION OF DRAWING(S) - The figure shows the lipopolysaccharide expression of the macrophage or dendritic cells derived from the **MD-2** genetically engineered mouse. (Drawing includes non-English language text).

Dwg.3/5

L60 ANSWER 43 OF 50 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:232309 BIOSIS
DOCUMENT NUMBER: PREV200510021832
TITLE: Crystal structure of CD14 and its implications for lipopolysaccharide signaling.
AUTHOR(S): Kim, Jung- In; Lee, Chang Jun; Jin, Mi Sun; Lee, Cherl- Ho; Paik, Sang- Gi; Lee, Hayyoung [Reprint Author]; Lee, Jie-Oh
CORPORATE SOURCE: Chungnam Natl Univ, Inst Biotechnol, Taejon 305701, South Korea
hlee@cnu.ac.kr; jieoh.lee@kaist.ac.kr
SOURCE: Journal of Biological Chemistry, (MAR 25 2005) Vol. 280, No. 12, pp. 11347-11351.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Jun 2005
Last Updated on STN: 23 Jun 2005

AB Lipopolysaccharide, the **endotoxin** of Gram-negative bacteria, induces extensive immune responses that can lead to fatal septic shock syndrome. The core receptors recognizing lipopolysaccharide are CD14, TLR4, and MD-2. CD14 **binds** to lipopolysaccharide and presents it to the TLR4/**MD-2 complex**, which initiates intracellular signaling. In addition to lipopolysaccharide, CD14 is capable of recognizing a few other microbial and cellular products. Here, we present the first crystal structure of CD14 to 2.5 angstrom resolution. A large hydrophobic pocket was found on the NH2-terminal side of the horseshoe-like structure. Previously identified regions involved in lipopolysaccharide binding map to the rim and bottom of the pocket indicating that the pocket is the main component of the lipopolysaccharide-binding site. Mutations that interfere with lipopolysaccharide signaling but not with lipopolysaccharide binding are also clustered in a separate area near the pocket. Ligand diversity of CD14 could be explained by the generous size of the pocket, the considerable flexibility of the rim of the pocket, and the multiplicity of grooves available for ligand binding.

IT Major Concepts
Toxicology; Biochemistry and Molecular Biophysics
IT Diseases
septic shock: bacterial disease
Shock, Septic (MeSH)
IT Chemicals & Biochemicals
CD14; TLR4; MD-2; lipopolysaccharide: toxin

L60 ANSWER 44 OF 50 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:530487 BIOSIS
DOCUMENT NUMBER: PREV200510324002
TITLE: Upregulation of plasma endothelin-1 (ET-1) levels and CD14

expression on peripheral blood monocytes in healthy children chronically exposed to urban air pollution.
AUTHOR(S): Calderon-Garciduenas, Lilian [Reprint Author]; Romer, Lina; Barragan, Gerardo; Reed, William
CORPORATE SOURCE: Univ Montana, Missoula, MT 59812 USA
SOURCE: FASEB Journal, (MAR 4 2005) Vol. 19, No. 4, Suppl. S, Part 1, pp. A489.
Meeting Info.: Experimental Biology 2005 Meeting/35th International Congress of Physiological Sciences. San Diego, CA, USA. March 31 -April 06, 2005. Amer Assoc Anatomists; Amer Assoc Immunologists; Amer Physiol Soc; Amer Soc Biochem & Mol Biol; Amer Soc Investigat Pathol; Amer Soc Nutr Sci; Amer Soc Pharmacol & Expt Therapeut; Int Union Physiol Sci.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Dec 2005
Last Updated on STN: 1 Dec 2005

AB Air pollution produces adverse health effects including systemic inflammation.Mexico City (MC) children are chronically exposed to high levels of ozone (03) and particulate matter (PM). **Endotoxins** are a significant constituent of MC PM 10 (59EU/mg) and PM2.5 (12 EU/mg). CD14 is a leucine-rich glycoprotein thatfunctions together with lipopolysaccharide-**binding** protein, **MD2** and toll-likereceptor 4 to initiate inflammatory responses to **endotoxin.** ET-1 is a vaso- and bronchoconstrictor rapidly produced by the lung following inhalation of 03 and PM. The objectives of this study were to determine whether MC children exhibit systemic responses to pollutant exposure by enhanced expression of CD 14 on circulating monocytes and upregulation of the ET-1. CD14 expression was assessed on peripheral blood monocytes by flow cytometry, and ET-I by ELISA, in acohort of 82 clinically healthy children aged 9 2 years (MC 59, Controls 23).Controls were age/gender matched children from Polotitlan, a town with low levels of air pollution. All children had unremarkable clinical histories. MC children exhibited a significant upregulation of CD14 (78.5 +/- 8.9 v 57.9 +/- 3.3, p=0.0012), and ET-1 (69.8 +/- 2.7 v 39.8 +/- 1.9 pg/ml p=< 0.0001) compared to children from Polotitlan. This finding suggests that monitoring CD14 and ET-1 in children could be used to follow environmental exposures to air pollution. Funded by KO1 NS046410-01A1.
IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Blood and Lymphatics (Transport and Circulation); Biochemistry and Molecular Biophysics
IT Parts, Structures, & Systems of Organisms
plasma: blood and lymphatics; lung: respiratory system; peripheral blood monocyte: immune system, blood and lymphatics; monocyte: immune system, blood and lymphatics, circulating
IT Chemicals & Biochemicals
particulate matter; ozone; toll-like receptor 4; MD2; endothelin-1: regulation; CD14: expression, regulation

L60 ANSWER 45 OF 50 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:561525 BIOSIS
DOCUMENT NUMBER: PREV200510341097
TITLE: Conserved mechanisms of signal transduction by Toll and Toll-like receptors.

AUTHOR(S): Gangloff, Monique; Weber, Alexander N. R.; Gay, Nicholas J. [Reprint Author]
CORPORATE SOURCE: Univ Cambridge, Dept Biochem, 80 Tennis Court Rd, Cambridge CB2 1GA, UK
njg11@mole.bio.cam.ac.uk
SOURCE: Journal of Endotoxin Research, (2005) Vol. 11, No. 5, pp. 294-298.
ISSN: 0968-0519.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Dec 2005
Last Updated on STN: 7 Dec 2005

AB In recent years, considerable progress has been made towards understanding the mechanism by which **endotoxin** is detected by the cells of the immune system. Lipopolysaccharides are extracted in a soluble form by the serum LPS **binding** protein and then transferred sequentially to the extrinsic membrane protein CD14 and the co-receptor **complex** TLR4/**MD-2**. Our modelling studies suggest that acyl chains of lipid A are buried within the hydrophobic core of **MD-2** and this induces cross-linking of the two TLR4/**MD-2 complexes**, an event that is required to trigger signal transduction. We also propose that, by analogy with the Drosophila Toll receptor, the mechanism of signal transduction is likely to be complex and to involve concerted protein conformational changes. In particular, we propose that receptor-receptor interactions mediated by juxtamembrane sequences play a critical role.

IT Major Concepts
Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis)

IT Parts, Structures, & Systems of Organisms
serum: blood and lymphatics; immune system: immune system

IT Chemicals & Biochemicals
lipopolysaccharide; **endotoxin**; toll-like receptor-4; CD14; lipid A; lipopolysaccharide binding protein; Toll receptor; MD-2: hydrophobic core; toll-like receptor-4/**MD-2 complex**

L60 ANSWER 46 OF 50 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:90089 BIOSIS
DOCUMENT NUMBER: PREV200500087944
TITLE: Interaction of soluble form of recombinant extracellular TLR4 domain with **MD-2** enables lipopolysaccharide **binding** and attenuates TLR4-mediated signaling.
AUTHOR(S): Hyakushima, Naoki; Mitsuzawa, Hiroaki; Nishitani, Chiaki; Sano, Hitomi; Kuronuma, Koji; Konishi, Masanori; Himi, Tetsuo; Miyake, Kensuke; Kuroki, Yoshio [Reprint Author]
CORPORATE SOURCE: Sch MedDept BiochemChuo Ku, Sapporo Med Univ, South 1,West 17, Sapporo, Hokkaido, 0608556, Japan
kurokiy@sapmed.ac.jp
SOURCE: Journal of Immunology, (December 1 2004) Vol. 173, No. 11, pp. 6949-6954. print.
ISSN: 0022-1767 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Mar 2005
Last Updated on STN: 2 Mar 2005

AB TLRs have been implicated in recognition of pathogen-associated molecular patterns. TLR4 is a signaling receptor for LPS, but requires MD-2 to

respond efficiently to LPS. The purposes of this study were to examine the interactions of the extracellular TLR4 domain with MD-2 and LPS. We generated soluble forms of rTLR4 (sTLR4) and TLR2 (sTLR2) lacking the putative intracellular and transmembrane domains. sTLR4 consisted of Glu24-Lys631. **MD-2 bound** to sTLR4, but not to sTLR2 or soluble CD14. BIAcore analysis demonstrated the direct **binding** of sTLR4 to **MD-2** with a dissociation constant of KD = 6.29 x 10-8 m. LPS-**conjugated** beads precipitated **MD-2**, but not sTLR4. However, LPS beads coprecipitated sTLR4 and MD-2 when both proteins were coincubated. The addition of sTLR4 to the medium containing the MD-2 protein significantly attenuated LPS-induced NF-kappaB activation and IL-8 secretion in wild-type TLR4-expressing cells. These results indicate that the extracellular TLR4 domain-**MD-2 complex** is capable of **binding** LPS, and that the extracellular TLR4 domain consisting of Glu24-Lys631 enables **MD-2 binding** and LPS recognition to TLR4. In addition, the use of sTLR4 may lead to a new therapeutic strategy for dampening **endotoxin**-induced inflammation.

IT Major Concepts
Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis); Membranes (Cell Biology)

IT Diseases
inflammatory disease: immune system disease, chemically-induced

IT Chemicals & Biochemicals
MD-2 protein; **endotoxin**; lipopolysaccharide: binding; nuclear factor kappa-B; toll-like receptor [TLR]; toll-like receptor-2 [TLR2]; toll-like receptor-4 [TLR4]: mediated signaling

L60 ANSWER 47 OF 50 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:462976 BIOSIS
DOCUMENT NUMBER: PREV200400465298
TITLE: Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4.
AUTHOR(S): Palsson-McDermott, Eva M. [Reprint Author]; O'Neill, Luke A. J.
CORPORATE SOURCE: Dept Biochem, Univ Dublin Trinity Coll, Dublin, 2, Ireland palssone@tcd.ie
SOURCE: Immunology, (October 2004) Vol. 113, No. 2, pp. 153-162. print.
CODEN: IMMUAM. ISSN: 0019-2805.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Dec 2004
Last Updated on STN: 1 Dec 2004

AB An understanding of lipopolysaccharide (LPS) signal transduction is a key goal in the effort to provide a molecular basis for the lethal effect of LPS during septic shock and point the way to novel therapies. Rapid progress in this field during the last 6 years has resulted in the discovery of not only the receptor for LPS - Toll-like receptor 4 (TLR4) - but also in a better appreciation of the complexity of the signalling pathways activated by LPS. Soon after the discovery of TLR4, the formation of a receptor **complex** in response to LPS, consisting of dimerized TLR4 and **MD-2**, was described.
Intracellular events following the formation of this receptor complex depend on different sets of adapters. An early response, which is dependent on MyD88 and MyD88-like adapter (Mal), leads to the activation of nuclear factor-kappaB (NF-kappaB). A later response to LPS makes use

of TIR-domain-containing adapter-inducing interferon-beta (TRIF) and TRIF-related adapter molecule (TRAM), and leads to the late activation of NF-kappaB and IRF3, and to the induction of cytokines, chemokines, and other transcription factors. As LPS signal transduction is an area of intense research and rapid progress, this review is intended to sum up our present understanding of the events following LPS binding to TLR4, and we also attempt to create a model of the signalling pathways activated by LPS.

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Immune System (Chemical Coordination and Homeostasis); Infection

IT Diseases
septic shock: bacterial disease
Shock, Septic (MeSH)

IT Chemicals & Biochemicals
CD14; MD-2; MyD88; MyD88-like adapter; TIR-domain-containing adapter-inducing interferon-beta; TRIF-related adapter molecule; Toll-like receptor-4; chemokine; cytokine; lipopolysaccharide: **endotoxin**; nuclear factor-kappa-B; transcription factor

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ACCESSION NUMBER: 2002:599180 BIOSIS
DOCUMENT NUMBER: PREV200200599180
TITLE: Response to Neisseria gonorrhoeae by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling.
AUTHOR(S): Fichorova, Raina N.; Cronin, Amanda O.; Lien, Egil; Anderson, Deborah J.; Ingalls, Robin R. [Reprint author]
CORPORATE SOURCE: Department of Infectious Diseases, Boston Medical Center, Boston University School of Medicine, 650 Albany Street, Boston, MA, 02118, USA
ringalls@bu.edu
SOURCE: Journal of Immunology, (March 1, 2002) Vol. 168, No. 5, pp. 2424-2432. print.
CODEN: JOIMA3. ISSN: 0022-1767.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Nov 2002
Last Updated on STN: 20 Nov 2002

AB Toll-like receptors (TLRs) have recently been identified as fundamental components of the innate immune response to bacterial pathogens. We investigated the role of TLR signaling in immune defense of the mucosal epithelial cells of the lower female genital tract. This site provides first line defense against microbial pathogens while remaining tolerant to a complex biosystem of resident microbiota. Epithelial cells derived from normal human vagina, ectocervix, and endocervix expressed mRNA for TLR1, -2, -3, -5, and -6. However, they failed to express TLR4 as well as **MD2**, two essential components of the receptor **complex** for LPS in phagocytes and endothelial cells. Consistent with this, endocervical epithelial cells were unresponsive to protein-free preparations of lipooligosaccharide from Neisseria gonorrhoeae and LPS from Escherichia coli. However, they were capable of responding to whole Gram-negative bacteria and bacterial lysates, as demonstrated by NF-kappaB activation and proinflammatory cytokine production. The presence of soluble CD14, a high-affinity receptor for LPS and other bacterial ligands, enhanced the sensitivity of genital tract epithelial cells to both low and high concentrations of bacteria, suggesting that soluble CD14 can act as a coreceptor for non-TLR4 ligands. These data demonstrate that the response to N. gonorrhoeae and other Gram-negative bacteria at the

mucosal surface of the female genital tract occurs in the absence of **endotoxin** recognition and TLR4-mediated signaling.

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Infection; Reproductive System (Reproduction)

IT Parts, Structures, & Systems of Organisms
cervicovaginal epithelial cells: reproductive system; ectocervix: reproductive system; endocervix: reproductive system; female genital tract: reproductive system; vagina: reproductive system

IT Diseases
Neisseria gonorrhoeae infection: bacterial disease, reproductive system disease/female
Neisseriaceae Infections (MeSH)

IT Chemicals & Biochemicals
CD14; LPS [lipopolysaccharide]; MD2; NF-kappa-B [nuclear factor-kappa-B]; Toll-like receptor 1 mRNA [Toll-like receptor 1 messenger RNA]; Toll-like receptor 2 mRNA [Toll-like receptor 2 messenger RNA]; Toll-like receptor 3 mRNA [Toll-like receptor 3 messenger RNA]; Toll-like receptor 4; Toll-like receptor 5 mRNA [Toll-like receptor 5 messenger RNA]; Toll-like receptor 6 mRNA [Toll-like receptor 6 messenger RNA]; lipooligosaccharide; proinflammatory cytokines

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ACCESSION NUMBER: 2003:28030 BIOSIS
DOCUMENT NUMBER: PREV200300028030
TITLE: Lipopolysaccharide modulation of normal enterocyte turnover by toll-like receptors is mediated by endogenously produced tumour necrosis factor alpha.
AUTHOR(S): Ruemmele, F. M. [Reprint Author]; Beaulieu, J. F.; Dionne, S.; Levy, E.; Seidman, E. G.; Cerf-Bensussan, N.; Lentze, M. J.
CORPORATE SOURCE: Paediatric Gastroenterology, Hopital Necker-Enfants Malades, INSERM EMI 0212, University Paris V, 149, Rue de Sevres, F-75743, Paris Cedex 15, France
ruemmele@necker.fr
SOURCE: Gut, (December 2002) Vol. 51, No. 6, pp. 842-848. print.
ISSN: 0017-5749 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Jan 2003
Last Updated on STN: 1 Jan 2003

AB Background: Circulating levels of **endotoxin** (or lipopolysaccharide (LPS)) and anti-**endotoxin** antibodies are increased in patients with inflammatory bowel disease, supporting the hypothesis of a role for endogenous bacterial products in the pathogenesis of these disorders. Aim: The aim of this study was to analyse the direct effects of LPS on intestinal epithelial cell turnover. Methods and Results: LPS significantly inhibited growth of the human non-transformed immature crypt cell line (HIEC), whereas IEC-6 cell proliferation was stimulated by LPS. As LPS is a physiological inducer of tumour necrosis factor alpha (TNFalpha) in various cell systems and this cytokine exerted similar anti-proliferative (HIEC) or growth stimulatory (IEC-6 cells) effects, the study thus tested the hypothesis that endogenously produced TNFalpha in response to LPS mediates this growth modulatory effect in an autoparacrine/paracrine way. Therefore, during LPS stimulation, the biological activity of TNFalpha was blocked using neutralising anti-TNFalpha antibodies, as well as inhibitory, antagonistic antibodies directed against the p55 TNF receptor, signalling the antimitotic TNFalpha

effect in HIEC. Both experimental approaches completely abolished the growth modulatory effects of LPS in HIEC/IEC-6 cells. Production and secretion of TNFalpha by HIEC/IEC-6 cells in response to LPS was confirmed on mRNA and protein level by reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assay. LPS signalling was independent of CD14 in HIEC, as these cells lack this receptor. However, HIEC expressed TLR4 and **MD2** resulting in a fully functional signalling **complex** as demonstrated by RT-PCR, western blot, and immunofluorescence analyses. Conclusion: These results support the hypothesis that LPS induced changes of intestinal epithelial cell turnover may directly contribute to the pathogenesis of inflammatory epithelial cell lesions by endogenous TNFalpha production by enterocytes.

IT Major Concepts
Digestive System (Ingestion and Assimilation); Immune System (Chemical Coordination and Homeostasis)

IT Parts, Structures, & Systems of Organisms
enterocyte: digestive system, modulation, turnover; intestinal epithelial cells: digestive system

IT Chemicals & Biochemicals
CD14; MD2: expression; lipopolysaccharide [LPS]: signaling; p55 tumor necrosis factor receptor; toll-like receptor 4 [TLR4]: expression; tumor necrosis factor-alpha [TNF-alpha]: production, secretion

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ACCESSION NUMBER: 2001:527328 BIOSIS
DOCUMENT NUMBER: PREV200100527328
TITLE: Secreted MD-2 is a large polymeric protein that efficiently confers lipopolysaccharide sensitivity to Toll-like receptor 4.
AUTHOR(S): Visintin, Alberto; Mazzoni, Alessandra; Spitzer, Jessica A.; Segal, David M. [Reprint author]
CORPORATE SOURCE: Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892-1360, USA
dave_segal@nih.gov
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (October 9, 2001) Vol. 98, No. 21, pp. 12156-12161. print.
CODEN: PNASA6. ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Nov 2001
Last Updated on STN: 23 Feb 2002

AB Toll-like receptor 4 (TLR4), the principal signaling receptor for lipopolysaccharide (LPS) in mammals, requires the **binding** of **MD-2** to its extracellular domain for maximal responsiveness. MD-2 contains a leader sequence but lacks a transmembrane domain, and we asked whether it is secreted into the medium as an active protein. As a source of secreted MD-2 (sMD-2), we used culture supernatants from cells stably transduced with epitope-tagged human MD-2. We show that sMD-2 exists as a heterogeneous collection of large disulfide-linked oligomers formed from stable dimeric subunits and that concentrations of sMD-2 as low as 50 pM enhance the responsiveness of TLR4 reporter cells to LPS. An MD-2-like activity is also released by monocyte-derived dendritic cells from normal donors. When coexpressed, TLR4 indiscriminately associates in the endoplasmic reticulum/cis Golgi with different-sized oligomers of MD-2, and excess MD-2 is secreted into the medium. We conclude that normal and transfected cells secrete a soluble form of **MD-2** that **binds** with high

affinity to TLR4 and that could play a role in regulating responses to LPS and other pathogen-derived substances in vivo.

IT Major Concepts
Immune System (Chemical Coordination and Homeostasis); Infection

IT Parts, Structures, & Systems of Organisms
dendritic cell: immune system

IT Chemicals & Biochemicals
MD-2 protein: epitope-tagged, extracellular domain, leader sequence, polymeric, secretion; Toll-like receptor 4: signaling receptor; lipopolysaccharide: bacterial **endotoxin**; pathogen-derived substances

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